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Freedom from viable trichinae of frankfurters prepared under Federal meat inspection. BENJAMIN SCHWARTZ, U. S. Bureau of Animal Industry.

INTRODUCTION

During the past few months various articles have appeared in the daily press and in magazines relative to the danger of acquiring trichinosis from frankfurters and hamburgers. The possibility that human beings might acquire trichinosis from hamburgers, if the latter happen to contain pork, cannot be excluded for the reason that these products are frequently prepared hastily in restaurants and roadside stands. When such is the case a hamburger may have a superficially cooked appearance and still be raw or rare in the middle. Some persons actually prefer rare hamburgers, such a preference being fraught with considerable danger, if the meat from which these products are made happens to contain pork. Frankfurters, on the other hand, are processed products, being smoked and cooked in the packing house in order to insure good keeping qualities and, under Federal and equally competent State or local meat inspection, to render them safe from a trichinosis standpoint. While the meat industry cannot guarantee that ground pork, which might be admixed with ground beef and sold in the retail meat shop or elsewhere as hamburger meat, is free from trichinae, that industry has a definite responsibility as regards the freedom from viable trichinae of frankfurters that are released for sale to the public.

NUMBER OF FRANKFURTERS CONSUMED IN THE UNITED STATES

That frankfurters constitute a rather important item in the diet of the American people is evident from the following considerations.

According to the National Provisioner (vol. 100, no. 12, p. 17, 1939) more than 460,000,000 pounds of frankfurters were made by the American sausage industry in 1937, this production representing approximately 4,500,000,000 frankfurters of average size or a per capita consumption of about 35 frankfurters. Considering, therefore, the importance of the frankfurter in the diet of the average American, it is quite essential that these products be so processed as to insure their freedom from viable trichinae. That the processing under Federal meat inspection is adequate from this standpoint is evident from the data given in this paper.

COOKING OF FRANKFURTERS UNDER FEDERAL MEAT INSPECTION

Under Federal meat inspection frankfurters, in common with certain other sausages, are regarded as products that are customarily eaten by the consumer without cooking. Consequently, these products are processed by the manufacturer in a manner that is known to be effective in destroying the vitality of trichinae, the requirements in such cases under Federal meat inspection being exceedingly rigid. The processing in the case of frankfurters includes cooking of a sort which insures a temperature of not less than 137° F. in the center of the product. Only such cooking procedures are approved by Federal inspectors which insure in all individual products a temperature of not less than 137° F. In actual practice, however, temperatures in excess of 137° F. are commonly attained in frankfurters during the cooking process.

EXAMINATION OF FEDERALLY INSPECTED FRANKFURTERS FOR TRICHINAE

In the past 5 years several thousand samples of meat food products containing pork muscle tissue have been obtained from officially inspected establishments and examined for trichinae by the digestion technique in the laboratories of the Zoological Division of the U. S. Bureau of Animal Industry. Briefly, this technique consists in grinding the sample in a meat chopper and mixing it thoroughly with a digestive fluid composed of water, pepsin, and hydrochloric acid,[1] an adequate quantity of fluid being provided to insure thorough digestion. When kept in an incubator for a period of about 18 hours, the sample so treated is well digested, but the trichinae, if present, escape the action of the digestive fluid and sink to the bottom of the container. Trichinae which have become brittle by the action of curing ingredients and heat may be broken into fragments during digestion and thus escape detection. Such fragments are dead and, therefore, harmless. In the sediment of the digestive fluid living as well as whole dead trichinae can be recovered, following repeated sedimentation and washing which eliminate the undigested fat, connective tissue and small fragments.

Using the technique described above, there were examined from May 22, 1934, to June 9, 1939, 1,118 one-half pound samples of federally inspected frankfurters. These samples were obtained from 175 meat packing establishments located in 30 cities of 25 states, as follows: California, Colorado, Delaware, Illinois, Iowa, Kansas, Kentucky, Louisiana, Maryland, Massachusetts, Minnesota, Missouri, Nebraska, New York, New Jersey, Ohio, Oklahoma, Oregon, Pennsylvania, Rhode Island, Tennessee, Texas, Virginia, Washington, and West Virginia. Out of this total of nearly 1,200 samples, only 11 were found to contain trichinae, the worms recovered, as shown below, being only 1 or 2 per positive sample; in no case did the worms show evidence of viability. Six of the positive samples contained 1 dead trichina each, the remaining 5 positive samples containing 2 dead trichinae each. In all cases, except 1, the worms were completely uncoiled, resembling the number 6, pale in appearance, and motionless even when gradually warmed, these characteristics being indubitable evidence of complete loss of viability. In 1 sample, which contained a single trichina, the worm was coiled but did not respond to repeated heat stimulation. When subjected to heat, a live trichina uncoils slowly and begins moving actively, these movements persisting for some time after the stimulus has been removed. In the case of the single trichina referred to, no response to heat could be elicited, despite repeated trials, and the worm was considered as having lost its vitality completely. The failure of occasional dead trichinae to uncoil has been noted repeatedly in this laboratory following processing of various kinds, and such coiled but immobile worms have yielded consistently negative results so far as infection is concerned when fed to white rats.

CONCLUSIONS

In the light of the data presented in this paper, it is evident that frankfurters prepared under Federal meat inspection requirements are adequately processed from the standpoint of trichina destruction, since only 11 out of 1,118 samples examined (0.9 per cent) contained dead trichinae, the maximum number in any one sample being 2; from the remaining 1,107 samples no trichinae were recovered. These facts demonstrate conclusively, therefore, that processing as conducted under Federal meat inspection produces frankfurters that are safe for human consumption even without subsequent cooking by the consumer. As a matter of common practice,

[1] For digesting a one-half pound sample, the digestive fluid was made up as follows: Scale or spongy pepsin U.S.P., 5 to 6 gm; hydrochloric acid, 10 cc; water, 600 cc.

however, frankfurters are generally boiled, fried, or roasted. However, even if the cooking process in the home or elsewhere is inadequate from the standpoint of trichina destruction, the danger of acquiring trichinosis from these products is nonexistent, because of the cooking requirements in the course of processing enforced by the Federal meat inspection service.

Intracutaneous tests for the detection of trichina infections experimentally and naturally acquired by swine. L. A. SPINDLER and S. X. CROSS, U. S. Bureau of Animal Industry.

The occurrence of trichina infections in swine and the importance of this parasite from the standpoint of human health (Schwartz, 1938, Jour. Amer. Vet. Med. Assoc. **92**, n. s. **45** (3): 317–337) has led to a number of investigations to determine the possibility of developing a test that can be applied to swine prior to slaughter to detect the presence of this infection. Such a test, if proven entirely reliable and applied to all hogs before slaughter, would probably aid materially in controlling the spread of trichinae to humans and to swine, provided carcasses of infected animals were processed to destroy trichinae or so disposed of as to eliminate the consumption of infected raw meat by human beings and swine.

In this connection Schwartz and McIntosh (1929, Jour. Parasitol. **16**: 104–105) applied the intracutaneous test (Bachman, 1928, Jour. Prev. Med. **2** (2): 169–173) for the detection of trichina infections to a small number of pigs experimentally infected with these worms. The reactions obtained were considered by these authors as specific, except in the case of one host animal. However, in subsequent investigations involving the testing of hogs in a large abattoir, Schwartz, McIntosh, and Mitchell (1930, Jour. Parasitol. **17**: 114) considered the test as being unsatisfactory. On the other hand, Augustine and Theiler (1932, Parasitol. **24** (2): 60–86) concluded from intracutaneous tests on 70 pigs (5 experimentally infected, 1 naturally infected and 64 uninfected) that the test furnishes an accurate and reliable method of detecting trichina infections in living swine. More recently, Otto (1938, Univ. Md. Agr. Expt. Sta., 51. Ann. Rept., p. 44) considered the test specific on the basis of tests involving animals experimentally infected.

In the light of the need for a reliable practical test for the detection of trichina infections in swine, investigations were undertaken to further develop the intracutaneous test for application to swine under conditions existing in abattoirs. It is the purpose of this paper to summarize briefly part of the data collected from May 1937 to December 1938. These data involve 5,274 tests made with 55 different trichina antigens on 1,493 swine.

EXPERIMENTAL METHODS

Test animals.—For the purpose of this report, animals tested are considered as comprising 4 groups. Groups A and C consist of 389 swine of various ages reared for experimental purposes at Beltsville, Maryland. Of these, 95 comprising group A had been given from 1 to 3 experimental infections with known numbers of trichina larvae in pork or rabbit muscle tissue one month to a year prior to the application of the intracutaneous tests. The remaining 294 animals comprising group C had not been experimentally infected and served, therefore, as controls.

Groups B and D consist of 1,104 garbage-fed and grain-fed hogs of various ages coming to slaughter at certain abattoirs. Of these, 54 comprising group B were naturally infected with trichinae and the remaining 1,050 comprising group D were uninfected, as determined post mortem by the digestion of the diaphragms.

Method of preparing antigens.—Antigens were prepared from trichina larvae obtained by peptic digestion of flesh of experimentally infected swine or rabbits. The

decapsulated larvae were first washed repeatedly in warm tap water and then passed through silk bolting cloth to free them of host tissue; only larvae free from host tissue were used in preparing antigens. Methods of preparing the 55 antigens used are too extensive to recount in detail in this paper. However, the following brief summary outlines these methods.

Certain antigens were prepared from dried larvae, others from live decapsulated larvae. The larvae (either living or dried) were first triturated and then extracted (1) in Coca's solution following procedures outlined by Bachman (*loc. cit.*); (2) in buffered and in unbuffered sterile physiologic saline; (3) in bovine serum; (4) in immune and in nonimmune swine serum; (5) in 60 per cent glycerine; (6) in Tyrode's solution; (7) suspended in physiologic saline and dialyzed into (a) distilled water, (b) physiologic saline, and (c) bovine serum.

There were used also as antigens excretory products of live larvae that had been kept in physiologic saline; in bovine serum; in immune and in nonimmune swine serum. These excretory products were also dialyzed into saline and into water, and then used as antigens. In addition, extracts of larvae (dried and undried) were fractionated and the fractions tested. Antigens tested had varying degrees of pH ranging from slightly acid to slightly alkaline.

Technic of making tests.—In certain tests animals were restrained and 0.1 to 0.2 cc of the antigen in dilutions of 1: 1,000, for the most part, were injected intracutaneously along the midline of the abdomen or along the inner surfaces of the thighs. In certain other tests antigens were introduced into skin at the base of the tail by means of an instrument having three lancet-like points arranged in the form of a triangle which was dipped into antigen before each test.

Control injections of physiologic saline made up in some cases in fresh distilled water and in other cases in tap water were used in some tests; Coca's solution was used as a control in other tests.

As many as 10 antigens were tested simultaneously on each animal; generally, however, 4 to 9 antigens and 1 control diluent were injected. In all cases the animal was slaughtered after the reactions were read; in no case was an animal used more than once.

Due to the extensive nature of the data, results from tests involving the various antigens will be considered as a unit in this report. It is intended to summarize in a later publication reactions obtained with various antigens.

Time of reading reactions.—Ante-mortem readings of reactions were made at intervals of 10, 15, 20, and in some tests 30, 45, 60, 120, and 240 minutes. In some cases readings were taken also as late as 48 hours following intracutaneous injections of antigens. As a routine procedure, the readings were also made on reactions after post-mortem scalding.

In the case of "stab tests" reactions could be read only following post-mortem scalding due to the thickness of the superficial layers of skin at the site of the scratches (base of tail). With these exceptions, data presented in this paper are based on ante-mortem readings made 15 to 20 minutes following injection and for purposes of this report data from all the readings will be considered as a unit.

Nature of reactions.—In the case of tests involving antigens injected intracutaneously, reactions considered positive generally appeared within 5 to 10 minutes after injection and reached a maximum intensity 15 to 20 minutes after injection. They generally consisted of a more or less blanched wheal at the site of injection surrounded by a bluish-red zone. However, in some cases the bluish-red area appeared without a distinct wheal but with a central anemic area; in other cases the reaction occurred as a flat, solid, bluish-red area without wheal. Reactions of these types have been commonly observed following intracutaneous injections of antigens in young pigs maintained in clean cement-floored pens and experimentally

infected with trichinae 4 to 6 weeks prior to the tests. As a practical procedure in these tests, reactions not typically positive were classed as doubtful. In addition, reactions consisting of a solid pinkish to red color with or without noticeable pufiness at the site of injection were classed as doubtful. In the case of reactions read following post-mortem scalding, those consisting of a circumscribed reddish pink area with a darker periphery were considered positive. Reactions classed as doubtful were those in which the color was pale and the area of inflammation indefinite.

For purposes of this report, two classes of reactions will be considered, namely, positive and doubtful. In case no reaction occurred at the site of injection of antigen, the results were considered "Negative."

In order to determine whether the swine tested were infected with trichinae as well as the extent of the infections, estimations were made by the digestion technic of the number of larvae present in either the entire diaphragm or a portion of the diaphragm of each animal tested. In the case of approximately one-half the animals tested in abattoirs, diaphragm samples were obtained which weighed 100 grams or more, each; in the case of the remaining animals the entire diaphragm was digested.

RESULTS OF TESTS

Results of 5,274 tests are summarized in the accompanying table. While no detailed discussion of the data will be given, it is considered desirable to call attention to some of the more outstanding features of the data.

Tests on infected hogs.—The most outstanding feature of the total series of 550 tests on 149 infected animals is the fact that in 29.8 per cent (average) of the tests no reactions occurred. Moreover, in only 48.7 per cent (average) of the tests were the reactions classed as positive; in 22.5 per cent the reactions were classed as doubtful (Table 1).

From an examination of the data pertaining to each of the two groups of infected swine tested, it can be seen that reactions obtained in tests on group A hogs (experimentally infected) were somewhat more reliable than in the case of those in group B (naturally infected). This is illustrated by the fact that in 401 tests on 95 infected group A animals 58.4 per cent of the reactions were definitely positive whereas in group B only 21.4 per cent of the 149 tests on infected animals produced positive reactions. Moreover, 53 per cent of tests on group B infected animals and 21.1 per cent of tests involving group A infected animals were entirely negative.

Tests on uninfected swine.—In the total series of 4,724 tests on 1,344 animals considered free of trichinae on the basis of diaphragm digestions, 77.6 per cent were negative. Typically positive reactions, however, occurred in 8.6 per cent (average) of the tests. In the case of group D swine, positive reactions occurred in 9.7 per cent whereas only 1.5 per cent of group C animals reacted positive.

It is interesting to note in this connection that 0.2 per cent of the control injections of 0.8 per cent saline or Coca's solution produced positive reactions on uninfected animals.

DISCUSSION

Findings herein reported indicate that the intracutaneous tests used for the detection of trichina infections in swine as applied under the conditions of these investigations failed to show that the test can be relied upon to detect all trichina-infected swine. Complete failure of various antigens to produce reactions in 29.8 per cent (average) of tests involving infected animals is of utmost importance from a practical standpoint. It was observed that a number of these nonreacting animals were heavily infected with trichinae. Failure of an infected animal to react to intracutaneous injections of trichina antigens was reported by Schwartz and McIntosh (*loc. cit.*) in the case of 1 of 8 pigs experimentally infected; this animal

TABLE 1.—*Results of intracutaneous tests for detection of trichina infections in swine*

Designation of animals[a]	Number swine tested	Number tests made	Reactions produced by test antigens						Reactions produced by control diluents					
			Positive		Negative		Doubtful		Positive		Negative		Doubtful	
			Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
Group A (infected)	95	401	236	58.4	86	21.1	87	20.5	.....	.....	92	96.8	3	3.2
Group B (infected)	54	149	32	21.4	78	53.0	39	25.6	.....	.....	50	92.6	4	7.4
Total or average	149	550	268	48.7	164	29.8	126	22.5	.....	.....	142	95.3	7	4.6
Group C (non-infected)	294	558	2	1.5	511	91.5	45	8.0	.....	.....	290	98.7	4	1.3
Group D (non-infected)	1,050	4,166	407	9.7	3,153	75.8	606	14.5	3	0.3	986	93.9	61	5.8
Total or average	1,344	4,724	409	8.6	3,664	77.6	651	13.8	3	0.2	1,276	95.0	65	4.8
Grand total	1,493	5,274												

[a] Group A, experimentally infected animals; group B, naturally infected animals tested at slaughter houses; group C, non-infected animals raised at Beltsville, Md.; group D, non-infected animals tested in slaughter houses.

was more heavily infected than the others tested. Wilkening (1937, Inaugural Dissertation, Hanover) found that 1 of 2 infected swine failed to give positive reactions to injections of trichina antigen. Although Augustine and Theiler (*loc. cit.*) did not find any infected animals that failed to react, they did report that intracutaneous injections of antigen in the case of 2 of 4 experimental pigs heavily infected failed to elicit more than weak positive reactions. Various investigators have occasionally reported that intracutaneous tests failed to produce positive reactions in humans known to be infected with trichina. For example, McCoy and Miller (1931, Jour. Parasitol. **18**: 123–124) reported that positive reactions were obtained in only 87 per cent of intracutaneous tests on proved human cases of trichinosis. McCoy, Miller, and Friedlander (1933, Jour. Immunol. **24** (1): 1–23) estimated that about 10 per cent of all persons ill with trichinosis fail to give a positive skin test. Kaljus (1936, Puerto Rico Jour. Trop. Med. **11** (4): 767–789) reported that he obtained positive reactions in only 74 per cent of the tests on known cases of trichina infections.

Perhaps of lesser importance from the standpoint of the tests is the 8.6 per cent positive reactions in uninfected animals. These findings are somewhat similar to those of other investigators. Schwartz, McIntosh, and Mitchell (*loc. cit.*) found that 9 of a series of 487 uninfected abattoir pigs tested with trichina antigen gave strong positive reactions. Augustine and Theiler (*loc. cit.*) reported nonspecific reactions in 3 of 21 pigs tested in Colombia and in 11 of 23 animals tested in Panama; trichina infections are considered to be rare or absent in pigs in those countries.

In the case of intracutaneous tests for the detection of trichina infections in humans, McCoy, Miller, and Friedlander (*loc. cit.*) reported that positive results were often encountered in tests on uninfected individuals. Kaljus (*loc. cit.*) obtained positive results in 5 per cent of uninfected persons tested.

Several explanations may be advanced to account for the occurrence of positive reactions in uninfected swine in these tests as follows:

(1) Sensitivity to trichina proteins resulting from an infection with trichinae which had not progressed to the point where the larvae would withstand artificial digestion and would not, therefore, be detected by the digestion technique. Augustine and Theiler found that their experimental animals sometimes became sensitive to intracutaneous injections of trichina antigen as early as 10 days after experimental infection. It has been observed by the writer that trichina larvae of that age will not survive artificial digestion (unpublished data).

(2) Sensitivity acquired as a result of consuming over a long period scraps of pork containing nonviable trichinae. In this connection weak positive reactions to intracutaneous tests were observed by the writers in pigs fed cooked trichinous meat over a period of several weeks (unpublished data).

(3) Sensitivity induced by injection of anti-hog-cholera serum obtained from blood of animals infected with trichinae. In this connection Schoenning and Creech (1935, Jour. Agric. Research **50** (1): 71–79) demonstrated that subcutaneous injection of 30 cc of anti-swine-erysipelas serum (equine) resulted in a transfer of agglutinins which could be demonstrated in the blood of injected pigs.

(4) Sensitivity occasioned by infestation with closely related parasites such as whipworms. That this will not serve to account for all false positive reactions is indicated by the fact that positive reactions were observed in these tests in trichina-free animals that had been raised under conditions that precluded exposure to infection with whipworms. Kaljus found that whipworm infestation in human beings did not influence result of intracutaneous tests with trichina antigens.

As stated previously results of tests herein reported indicate that the intracutaneous test as applied for the detection of trichina infections in swine is lacking

in specificity and cannot be relied upon to detect all infected animals. If an intracutaneous test for the detection of trichina infections in swine is to be used on a practical basis, it should detect all infected animals irrespective of the age and color or the age and degree of infection. Furthermore, reactions must be of such strength, distinctness, and clarity that they will under no circumstances be masked by wrinkles or pigmented areas in the skin or be confused with bruises, abrasions, or with any of the other abnormal conditions frequently found on the skin of swine coming to slaughter in abattoirs. In addition, the reactions must be so distinct and clear cut that they can be readily observed by trained inspectors.

In the light of facts set forth in this paper, it is evident that recommendations for use of the intracutaneous test for detection of trichina infections as a practical procedure cannot be safely made on the basis of tests involving small numbers of animals experimentally infected with trichinae.

Methods of determining the viability of *Balantidium coli* cysts. JOHN C. LOTZE, U. S. Bureau of Animal Industry.

The extensive literature on *Balantidium coli*, a ciliated protozoan occurring in the intestines of man and domestic swine, deals with studies on trophozoites, for the most part, comparatively little information being available on the cysts which are commonly regarded as the infective stage of the parasite. For developing proper control methods for porcine balantidiasis more definite information should be available relative to the resistance of the cysts to various environmental conditions. In order to secure this information there should be a reliable method for testing the viability of the cysts. A need also exists for determining the viability of cysts to be used in experimental infections involving the pig as a host. In the present investigation, a small laboratory animal was sought for the purpose of testing the viability of *B. coli* cysts from the pig.

Schumaker (1929, Science **70**: 384; 1930, Amer. Jour. Hyg. **12**: 341–365) showed that experimental infections with *B. coli* from the pig could be produced in white rats by injecting into various parts of the alimentary canal material containing trophozoites. This investigator obtained negative results by injecting cysts. On the other hand, Obitz (1931, Ztschr. Parasitenk. 3: 649–653) was able to infect wild rats by feeding them cysts of *Balantidium coli* from the pig. In the light of these investigations, a series of experiments were carried out at the Agricultural Research Center, Beltsville, Maryland, during February and March, 1939, to find a suitable method of testing the viability of *B. coli* cysts. Albino rats were used as experimental host animals.

Cysts used in the experiments herein described were obtained from fresh feces collected immediately after their elimination from the pig. Tap water having a temperature of 25° to 35° C. was used in washing the feces through screens, and the cysts were allowed to stand in the water overnight. On the following day, cysts were concentrated by centrifuging either in tap water for introduction into the stomachs of rats by means of a duodenal tube, or in Ringer's solution for injection outside the alimentary canal, as noted elsewhere in this paper. In all cases 2 cc of liquid containing the cysts were used.

TESTS INVOLVING CYSTS ADMINISTERED BY DUODENAL TUBE

In order to determine whether cysts of *B. coli* will become activated and in what part of the alimentary canal the activation occurs, approximately 20,000 cysts were administered into the stomach of each of 16 rats. Two rats were killed at each period of 1, 2, 4, 6, 8, 10, 24, and 48 hours after the introduction of cysts, and the alimentary canal was examined for the presence of cysts and trophozoites. The post-mortem findings are summarized in table 1.

TABLE 1.—*The distribution of inactivated cysts (c),[a] activated cysts (¢),[b] and trophozoites (t) of Balantidium coli in the alimentary canal of albino rats experimentally infected*

Rat. No.	Time after injection (hours)	Stomach	Upper small intestine	Lower small intestine	Cecum	Large intestine	Rectum (in fecal contents)
1	1	c	c	c ¢			
2		c	c	c ¢	c		
3	2	c	c	c ¢	c ¢		
4		c	c	c ¢	c ¢		
5	4	c	c	c ¢	c ¢	c ¢	
6		c	c	c ¢	c ¢	c ¢	
7	6			c ¢	c ¢ t	c ¢	
8				c ¢	c ¢ t	c ¢	
9	8				c ¢ t	c ¢ t	
10					c ¢ t	c ¢	
11	10				c ¢ t	c t	c ¢ t
12					c ¢ t	c ¢ t	c ¢ t
13	24				t	c t	c t
14					c t	c t	c
15	48				t	t	t
16					t	t	t

[a] Cysts of same appearance as in feces; [b] cysts showing active trophozoites.

As shown in table 1, activated cysts were recovered from the lower portion of the small intestine, the cecum, large intestine and rectum. Trophozoites were not found anterior to the cecum. In the case of rats 1 and 2, killed at the end of 1 hour, cysts were found not only in the stomach but throughout the length of the small intestine. Trophozoites were first observed in rats 7 and 8, killed 6 hours after infection; the trophozoites were apparently confined to the ceca of these host animals. In the case of rats killed at the end of 10 hours, activated and unactivated cysts as well as trophozoites were present in the soft feces contained in the rectum. The same condition was found in the rats killed at the end of 24 hours, with the exception that no activated cysts were recovered. In rats killed at the end of 48 hours, trophozoites only were found in the cecum, large intestine and rectum.

TESTS INVOLVING CYSTS INJECTED INTRAPERITONEALLY

In order to determine whether excystment would take place in the abdominal cavity of the rat, approximately 10,000 cysts were injected into the abdominal cavity of each of 14 rats. Four of these rats were killed at the end of 5 hours and 2 were killed at the end of 7 hours; of the rats remaining, 6 died at the end of 10 hours and 2 were killed at the end of 24 hours. The number of cysts (active and inactive) and trophozoites recovered postmortem are shown in table 2.

TABLE 2.—*Number of cysts and trophozoites of B. coli recovered from rats following intraperitoneal injection of cysts*

Number of rats examined	Time after injection (hours)	Estimated number of cysts recovered (average)	Estimated number of trophozoites found (average)
4	5	775	225
2	7	3,000	14
6	10	1,700	47
2	24	727	302

As shown in table 2, trophozoites were found in all the rats except one which died at the end of 10 hours. The injected material was found in small clumps adhering tightly to the viscera.

TESTS INVOLVING CYSTS INJECTED INTRADERMICALLY

Since it was found that excystment would take place in the abdominal cavity, 2 tests were made to determine if excystment would also occur when cysts were injected intradermically.

Test 1.—Approximately 10,000 cysts were injected intradermically in one site on the abdomen of each of 16 albino rats. These rats were killed and examined at intervals of 5, 7, 10, and 24 hours, respectively, as shown in table 3.

Test 2.—Approximately 16,000 cysts were injected into each of 4 sites in the skin of each of 4 rats. In each of 2 other rats, injections were made in 2 sites in the skin. One rat was killed at each period of 1, 5, 7, and 11 hours after injection. The average of the estimated number of cysts (active and inactive) and trophozoites recovered per single injection in rats killed at the end of the different time intervals is shown in table 3.

TABLE 3.—*Estimated number of cysts and trophozoites of B. coli recovered from site of intradermic injection of cysts*

	Number of rats used	Time after injection (hours)	Number of sites injected	Estimated number of cysts recovered per site (average)	Estimated number of trophozoites found per site (average)
Test 1. Approximately 10,000 cysts injected per site	4	5	1	6,550	559
	2	7	1	10,000	361
	6	10	1	4,750	172
	4	24	1	550	4
Test 2. Approximately 16,000 cysts injected per site	1	1	4	10,950	0
	1	3	2	19,200	6
	1	5	4	12,700	3
	1	7	4	12,150	675
	1	9	2	12,700	900
	1	11	4	10,250	337

Data given in table 3, as well as those given in table 2, demonstrate that factors responsible for activation of *B. coli* cysts are not confined to the alimentary canal, but are also present in the abdominal cavity and in the skin of the albino rat. In addition, it is indicated that some encysted trophozoites require longer periods of time for excystment than do others. The number of trophozoites recovered at various intervals were not directly proportional to the interval elapsing between injection and post-mortem examination.

DISCUSSION AND SUMMARY

Data from the tests herein described indicate that the viability of *Balantidium coli* cysts from the pig can be tested by introduction of the cysts into the alimentary canal of albino rats or by the intradermic and intraperitoneal injections of cysts into these hosts. Counts were not made of cysts and trophozoites in the alimentary canal, because the study was made primarily to determine where the cysts become activated and where excystment takes place. The available data show that the introduced material is carried through the alimentary canal in a relatively short time; in the case where rats were killed 10 hours after cysts were introduced into the

stomachs of the rat host, the activated cysts were present in the feces found in the rectum. Intradermic injections of cysts were easily made and the material so injected was less difficult to recover than when other methods of injection were used.

A new species of coccidium from cattle, with observations on its life history. JOHN F. CHRISTENSEN and DALE A. PORTER, U. S. Regional Animal Disease Research Laboratory, Auburn, Alabama.

During the course of a series of fecal examinations on a young Jersey calf, oöcysts of what appeared to be a new species of *Eimeria* were observed. This calf had been in isolation since its birth on October 9, 1938, and, with the exception of *Strongyloides papillosus*, no parasites were detected by triweekly fecal examinations from November 28 to December 20, when the new coccidium was found. The oöcysts recovered at that time possessed a peculiar, rough, heavily-mammillated wall, and were believed to be those of a distinctly new species. However, when these oöcysts were fed to a susceptible calf, oöcysts varying in wall structure from heavily mammillated to perfectly smooth and homogeneous were recovered. The latter were identical with certain smooth-walled oöcysts previously collected from the feces of other calves in the vicinity of Auburn, Alabama. Since both the rough- and smooth-walled oöcysts were similar in size, shape, color, and sporulation time, and formed an intergrading series as regards wall structure, it appeared that we were dealing with a single species, the oöcysts of which differed markedly from those of known species of coccidia from cattle. For this parasite the name *Eimeria auburnensis* is proposed.

Eimeria auburnensis, n. sp.

Unsporulated oöcysts.—Oöcysts 32 to 45.5 μ (average 38.4 μ) long by 20 to 25.5 μ (average 23.1 μ) in transdiameter; 10 to 100 consecutive oöcysts were measured from each of 8 host animals, dimensions being based on a total of 350. Shape typically elongated ovoidal, varying between subellipsoidal and markedly tapered, the broad end being the segment of a circle and the narrow end formed by gradual attenuation, 0.48 to 0.76 (average 0.60) as broad as long. Wall 1 to 1.5 μ in thickness, typically smooth, homogeneous, transparent, noticeably brownish-yellow in tint. In unstained specimens the micropyle is indicated by a thin, pale area over the narrow end, while in oöcysts impregnated with an iodine-eosin mixture in physiological saline the micropyle is seen to be a definite gap in the wall, covered with a narrow black line which possibly represents a flat operculum. In structure the wall, that forms a circular opening at the narrow end through which oöcyst membrane protrudes as a rounded lobe, varies from the typically transparent, homogeneous type (Fig. 1, B & E) to the unusual, semitransparent, heavily-mammillated type (Fig. 1, A & C); all intergradations occur between homogeneous and mammillated walls, with the smooth type greatly predominating in numbers and frequency of occurrence. The elevations in the wall appear to be yellowish, rounded granules embedded in a homogeneous matrix, which in stained specimens is observed to form an irregular lattice-work over the surface of the oöcyst; the color in the wall is usually brownish-yellow, varying from almost colorless to light brown. Early spherical sporont 18 to 23 μ (average 21.3 μ) in diameter.

Sporulation.—At room temperature freshly-discharged oöcysts sporulate completely within 48 to 72 hours after isolation into a drop of clean tap water. There is no residual body in the oöcyst, but in the sporocyst residual material occurs in the form of rounded masses or individual granules scattered along the inner lateral margins of the sporozoites.

Relationships.—The oöcysts of *E. auburnensis* are easily distinguished from those of *E. smithi* by greater mean size, more intense coloration, relatively nar-

rower and more elongated shape, and a tendency toward the development of a roughened or mammillated coat. They differ from oöcysts of *E. bukidnonensis* in having a thinner wall, paler brownish tint, greater transparency, shorter sporulation time (5 to 7 days in *E. bukidnonensis*), relatively narrower form, and tendency toward the formation of the mammillated coat. Oöcysts of *E. auburnensis* differ from those of *E. thianethi* in having smaller average size and thinner wall,

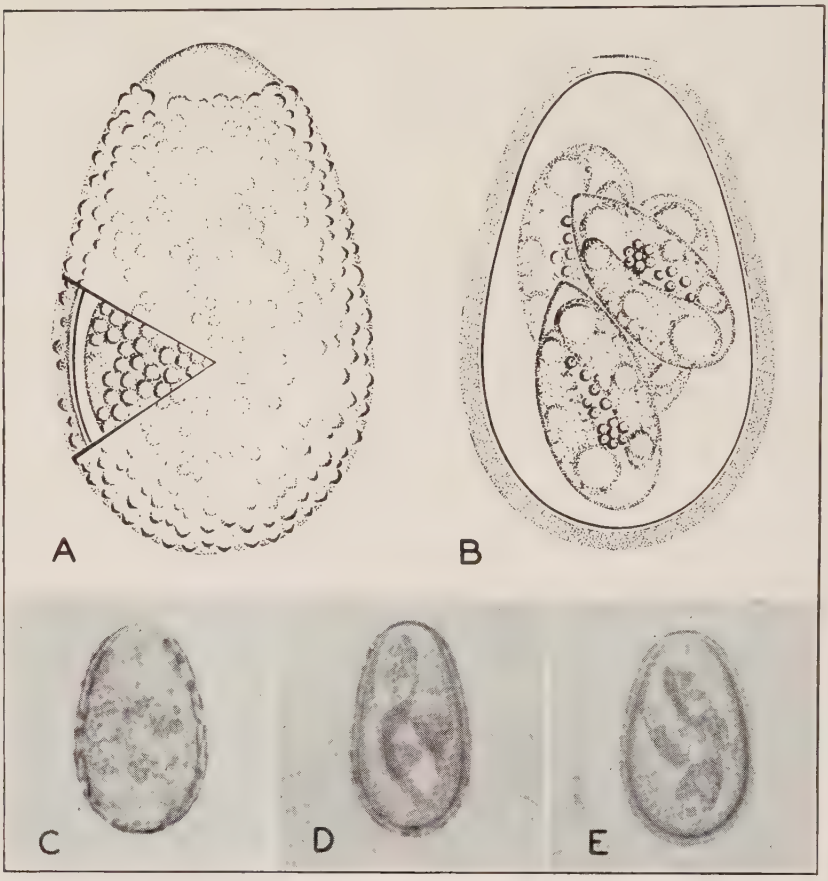

FIG. 1. *Eimeria auburnensis*, n. sp. A—Drawing of oöcyst with heavily-mammillated wall, with section removed to show structure of wall and portion of spherical sporont. ×1600. B—Drawing of oöcyst with smooth, homogeneous wall, showing 4 sporocysts, each containing 2 sporozoites and residual material. ×1600. C—Unsporulated oöcyst with heavily-mammillated wall, showing discontinuity of roughening over narrow end. D—Sporulated oöcyst with wall showing intermediate mammillation. E—Sporulated oöcyst with smooth, homogeneous wall.

and do not show the transverse striations reported for the oöcysts of *E. thianethi*.

Distribution.—Oöcysts of this species were found in fecal samples from 8 calves from Alabama, 1 calf from Maryland, and 2 calves from Montana.

As far as the writers were able to determine, the mammillated or rough-coated oöcysts as described above for *E. auburnensis* have not been previously observed. Since the described variants were found in pure infection, it was at first believed that they represented a new species of *Eimeria*. To determine their ability to

infect a susceptible host and to produce similar oöcysts in the feces, the following infection experiment was conducted.

As determined by dilution count, the calf in which the original infection was found discharged on December 20, 1938, 3,200 rough-coated oöcysts per cc of once-strained fecal sediment. A few similar oöcysts were passed on the following day, after which none was observed. In the first of these samples careful examination showed that while the oöcysts with extremely mammillated walls greatly predominated, there were a few in which the elevations in the wall were only moderately numerous or sparse; it was believed that these differences were within the limits of variation of a species.

The fecal sediment containing the oöcysts was stored at 4° to 8° C. in tap water without preservative, the low temperature together with the small amount of unavoidable putrefaction preventing sporulation. The oöcysts were sporulated in a shallow layer of 2 per cent potassium dichromate solution at room temperature during the week prior to dosage of the susceptible calf. On February 24, 1939, when the oöcysts were slightly more than 2 months old, 8,000 sporulated oöcysts were administered by pipette to a 2-weeks-old calf that had been fasted for 24 hours previous to dosage. This calf had been kept in isolation since the date of birth, and repeated examinations for coccidial oöcysts and helminth eggs were negative.

Feces passed during the night was collected every morning, and that passed during the day was collected in late afternoon. Representative quantities of these samples were mixed with water, strained once through a sieve having 60 meshes per linear inch, sedimented, and sugar flotations made. In this way there was but a slight possibility of missing infection indicated by oöcyst discharge. The behavior of the calf was normal throughout the infection with the exception of 5 days of profuse, watery, green diarrhea, accompanied by slight apathy on the part of the host, lasting from the 9th to the 13th day after the infective dose was given. There was no sign of oöcyst discharge until March 20, or the 24th day after infection, when the morning sample showed 319,000 oöcysts per cc of once-strained fecal sediment. The oöcyst count dropped to 54,000 by late afternoon, and gradually diminished to 7,500 by the 27th day of infection. Small numbers of oöcysts continued to be discharged during the next few weeks, but never in sufficient numbers to warrant a dilution count. Neither oöcysts of other species of coccidia nor helminth eggs were passed during the entire period of the experiment.

Measurements and observations of the oöcysts showed that the discharge represented a pure infection. The oöcysts were uniform in size, shape and color, forming normal curves of distribution. The most striking variation consisted of a complete series in wall development from the heavily-mammillated type, characteristic of oöcysts of the infective dose (Fig. 1, A & C), through all degrees of intermediate mammillation (Fig. 1, D), to those in which the wall was perfectly homogeneous and smooth (Fig. 1, B & E). The smooth-walled variants greatly predominated in numbers, however, indicating that they are possibly more characteristic of the species than the rough-walled type. That the smooth-walled oöcysts are more normal for the species is also indicated by the fact that we have observed them in natural infections of 8 calves near Auburn, Ala., while the rough-coated variants have been observed only twice, both findings representing relatively small numbers of oöcysts from calves housed in the same barn.

The smooth-walled, tapered, yellowish-brown variants have undoubtedly been observed by others. Marsh (personal communication) found these oöcysts in several calves in Montana and he expressed the view that they differed from other species of coccidia from cattle. Smith and Graybill (1918, Jour. Expt. Med. **28**: 89–108) in New Jersey were probably dealing with the smooth-walled variants of

E. auburnensis when they reported oöcysts measuring up to 41.8 μ. This is likewise probably true of Wilson and Morley (1933, Jour. Amer. Vet. Med. Assoc., **82**: 820–850) in Virginia, when they noted large oöcysts measuring 46.2 μ by 25.2 μ, and suggested that these forms did not conform to oöcysts of known species of bovine coccidia. Considering the ease with which we have found the smooth-walled variants in calves in Alabama, including the animal in which the original rough-coated oöcysts were found, it is likely that this species has been seen by many others; however, no one appears to have demonstrated its distinction from other species of bovine coccidia or noted the relationship to a rough-walled variant.

Gwéléssiany (1935, Ann. Parasitol. **13**: 338–341) described *Eimeria thianethi* on the basis of oöcysts found in the feces of bovines in the Erzo-Thianethi district of the U.S.S.R. Briefly, these oöcysts were described as follows: Shape, oval; color, dull yellow; length, 33.7 to 48.7 μ (average 42.6 μ); breadth, 26.2 to 33.7 μ (average 28.6 μ); ratio of breadth to length, 0.59 to 0.77 (average 0.66); wall about 2 μ in thickness and composed of 2 layers, the outer thin and homogeneous, the inner thick and showing distinct transverse striations; micropyle distinct in certain oöcysts. Oöcysts of this species were found in small numbers in only 5 out of 30? animals examined. Because of insufficient material, Gwéléssiany was unable to make a large number of measurements, to ascertain the presence or absence of residual bodies after sporulation, or to follow the development of oöcysts in culture. He placed emphasis in identifying oöcysts of *E. thianethi* upon the presence of transverse striations in the wall. Although oöcysts of *E. auburnensis* resemble those of *E. thianethi* in general shape, color and size, the complete absence of any suggestion of wall striations in the former strongly indicates that the two are distinct. Furthermore, the rounded elevations in the wall of the rough-coated variants of *E. auburnensis* could scarcely be interpreted as striations.

The writers have found oöcysts of all known species of bovine coccidia, with the exception of *E. thianethi*, in the feces of calves in the vicinity of Auburn, Alabama. As far as we have been able to determine, oöcysts of *E. thianethi* have not yet been reported from the United States.

Comparative counts of infected and noninfected erythrocytes in bovine anaplasmosis. CHARLES W. REES[1] and PAUL C. UNDERWOOD,[2] U. S. Bureau of Animal Industry.

In considering the question whether anaplasmata are protozoan organisms or reaction products of some kind, it would be helpful to know whether the erythrocytes become infected in some special part of the circulatory system. In malaria, for example, according to Schaudinn (1902, Arb. K. Gsndhtsamt. **19**(2): 169–250) the merozoites of *Plasmodium* probably gain access to the erythrocytes in the smaller capillaries where the blood flow is slowest. If anaplasmata have the power of locomotion they might gain access to the erythrocytes in much the same manner as *Plasmodium*. If, on the other hand, the anaplasmata are reaction products they might be expected to appear in the erythrocytes during erythropoiesis in the bone marrow. In malaria as in anaplasmosis and other diseases caused by parasites of the suborder Haemosporidiidea there is a carrier state following the acute attack; during the former the host appears to be in good health. However, it may be demonstrated by injection into a susceptible host of even a minute amount of blood from the carrier that the parasites have not disappeared from the blood. One of the major problems connected with these diseases arises from the fact that no practicable method has thus far been devised to detect the carrier state. For this purpose the injection with virulent blood of small inexpensive laboratory animals is of

[1] Transferred to National Institute of Health, April 16, 1939.
[2] Transferred to U. S. Bureau of Dairy Industry, October 16, 1937.

TABLE 1.—*Counts of Anaplasma marginale in bovine blood smears*

Animal designation	Date of inoculation (1934)	Date of examination (1934)	Stage of the disease (incubation (i); clinical (cl); carrier (c))	Status when examined (living (l); dead (d))	Anaplasmata per 1,000 erythrocytes				
					Peripheral blood	Bone marrow	Liver	Spleen	Lungs
126	Oct. 26	Oct. 26	i	l	0	0	0	0	0
126		Nov. 6	i	l	0	0	0	0	0
126		Nov. 16	cl[a]	l	56	60	60	40	59
126		Nov. 24	cl	d[b]	112	34	60	50	65
120	Sept. 20[c]	Oct. 10	cl	d[b]	147	48	110	.....	.....
124	Nov. 1	Dec. 2	cl	d[d]	149	105	.....	.....	.....
123	July 20	Oct. 2	c	l	1	4	4	.....	.....
122	July 6	Oct. 30	c	d[b]	0	1	0	2	6

[a] First day of reaction.
[b] Killed for pathology.
[c] Date of splenectomy of a carrier; the clinical period was a post splenectomy relapse.
[d] Died during night.

no avail because of the rigid host-parasite specificity that prevails. Serological diagnostic methods have likewise been unsuccessful. It occurred to the writers that in anaplasmosis there might be a focus of infection in the bone marrow or elsewhere within the circulatory system and, if so, erythrocytes taken from this focus might be found infected even when the parasites had apparently disappeared from the peripheral blood. The purpose of the present investigation was, therefore, (1) to find out something concerning the nature of anaplasmata and (2) to discover a method of detecting the infection in carriers.

MATERIALS AND METHOD OF PROCEDURE

The bovines used as subjects for the transmission of anaplasmosis were maintained at the Agricultural Research Center, Beltsville, Md. On these animals comparative counts were made of anaplasms occurring in peripheral blood and in blood from bone marrow, liver, spleen, and lungs. Stained smears from the sources mentioned were secured from (1) fatal cases post mortem, (2) carrier cases, and (3) other cases during the incubation and clinical stages of the disease. The bone marrow smears were taken from either the eleventh or the twelfth rib, about 6 inches from the articulation with the vertebral column, while the animals were under chloral hydrate anaesthesia. After exposing the rib the periosteum was interrupted with a one-inch incision and the edges retracted and clamped. An opening into the marrow was then made with a $\frac{1}{4}$-inch steel drill and the material drawn out with a glass pipette. For splenic and liver punctures the technique described by Sergent *et al.* (1924, Arch. Inst. Pasteur Algérie **2**(1): 1–146) was followed; a similar method was used for lung punctures.

DATA OBTAINED

The data obtained are recorded in table 1 which shows (1) that during the incubation period of the disease *Anaplasma* was not seen, (2) that during the clinical period anaplasms were no more plentiful and probably less plentiful in the bone marrow, liver, spleen and lungs than in the peripheral blood, and (3) that bodies resembling *Anaplasma* were found in the blood of carriers; in 1 case *Anaplasma*-like bodies were quite plentiful in the blood from the lungs. The data do not indicate that a focus of infection occurred that would serve as a source of material for diagnosing carrier cases. These data cast no light on the nature of anaplasmata.

COMMENTS

Had a focus of infection showing numerous anaplasmata as occurs during the clinical course of the disease been found in carriers, the results of the present paper would have been significant. If *Babesia bigemina* or *B. argentina* could be found in carriers, no matter how few in number, carrier cases of these infections could be diagnosed by direct microscopic examination, because piroplasms may be identified with certainty. With *Anaplasma,* however, even during the clinical period, this organism could not be positively identified if the organisms were as scanty during the clinical periods as either *Babesia bigemina* or *B. argentina.* Obviously then, a stain that would differentiate *Anaplasma* specifically, as mitochondria are differentiated by janus green or reticulocytes by brilliant cresyl blue, would be necessary in detecting carriers of anaplasmosis.

The effect of some halogenated hydrocarbons on the eggs of *Toxocara canis* (Nematoda). JOHN T. LUCKER, U. S. Bureau of Animal Industry.

INTRODUCTION

Lucker and Shaffer (1937, Vet. Med. **32**: 564–569) recently reported that dichloropentanes (a commercial mixture of isomers), carbon tetrachloride, ethylene dichloride, and chlorosol (a commercial mixture of 3 parts by volume of ethylene dichloride and 1 part carbon tetrachloride) were found in laboratory experiments to be lethal to eggs and preinfective larvae of horse strongyles; they also noted that chlorosol appeared to be lethal to ascarid eggs in horse feces. In initiating a search for chemicals which might be applied to the soil of kennel runways for the purpose of killing the eggs of the ascarids of dogs and foxes, it seemed logical to explore first the possibilities of these substances. The preliminary laboratory experiments are given in this paper.

After the paper referred to was published, the writer noted that ethylene chloride (synonymous with ethylene dichloride) had previously been reported to have a lethal action on horse strongyle eggs and larvae by Parnell (1937, Canad. Mining Jour. **58**: 242–246); this author also listed some other closely related lethal organic compounds.

MATERIALS AND METHODS

The eggs used in the experiments were obtained from the uteri of *Toxocara canis* from dogs. When the eggs had been freed from the large pieces of uterine tissue, they were concentrated and suspended in a few cc of water. In the first 3 experiments equal portions of the egg suspension, prepared on the day the worms were collected or before the eggs had undergone development, were added to the culture media. In experiment 4, the procedure was similar, but the suspension consisted of eggs which had been cultured in tap water at room temperature for 19 days.

The soil used in experiments 2, 3, and 4 was sterilized sandy clay loam. A layer of this soil, about 1 cm deep, was placed in small Stender dishes and enough water was added to dampen it. The surface area of the soil in the dishes used in all experiments was 14.5 sq. cm. A portion of the egg suspension was distributed on the surface of the soil with a pipette and a little dry soil was added to take up the excess moisture. The liquid chemicals were similarly distributed on the soil surface. The dishes were covered with lids grooved and ground to fit. As noted in the tables, the lids of some dishes were treated with petrolatum to seal in the chemical vapors; the procedure in this respect, in the aeration of the cultures, and in removing the petrolatum from the lids before replacing them on the dishes, was similar to that used by Lucker and Shaffer (*loc. cit.*). The soil was not, however, removed from the dishes during the period of aeration. The interval in which the dish was sealed or covered, previous to the aeration of its contents, may be regarded as the period of intensive exposure to the chemical vapors; particularly in soil treated with dichloropentanes, some residue of the chemical remained in the soil thereafter.

Eggs were isolated from the soil for examination by means of methods usually employed for this purpose. It was not considered necessary to prepare water suspensions of the eggs for dilution counts. Eggs adhering to the slide or coverslip were examined directly to determine their morphological or other status, and were counted at random without the exercise of any selection. In experiments 1, 2, and 3, only incidental attention was given to the vitality of the embryos in the eggs, since the object was merely to determine which, if any, of the chemicals had completely prevented development. In examining embryonated eggs from the soil preparations involved in experiment 4, the criteria of vitality were as follows:

Eggs containing vacuolated, deeply colored larvae which did not exhibit spontaneous movement were classified as dead; eggs containing larvae showing neither obvious morphological degeneration, not spontaneous movement, were tested by applying a heated glass rod to the under surface of the slide. In all experiments, only eggs with a fully formed shell were counted and examined.

EXPERIMENTAL DATA

Effect on development of eggs in water cultures.—In experiment 1, 4 cc of the egg suspension were added to 10 cc of tap water and a few drops of formalin in each of 3 small Petri dishes; 1 cc of chlorosol was added to one culture and 1 cc of dichloropentanes was added to a second. The third culture, to which 1 cc of water was added, served as a control. The dishes were covered with lids; no other provision was made to avoid evaporation or to confine the chemical vapors. The development of the eggs was observed at frequent intervals over a period of 9 days. The eggs in the control culture and in the culture containing dichloropentanes developed at about the same rate and in the same proportions, some eggs in each culture containing immature larvae on the 9th day. The protoplasm of most of the eggs developing in the culture with dichloropentanes had an unnatural dark color. A similar proportion of developing eggs was noted in the culture containing chlorosol, but by the ninth day none had advanced beyond the morula stage.

TABLE 1.—*Effect of chlorosol and dichloropentanes on development of Toxocara eggs in water cultures in experiment 1*

Culture No.	Chemical used	Period of culture	Number of eggs examined	Condition of eggs examined				Condition of larvae in embryonated eggs
				Undeveloped or partially developed		Embryonated		
		Days		*Number*	*Per cent*	*Number*	*Per cent*	
1	Chlorosol	37	519	398	76.7	121	23.3	Majority normal; remainder dark and of abnormal structure.[a]
2	Dichloropentanes	37	548	334	60.9	214	39.1	About 10 per cent normal; remainder dark and of abnormal structure.[a]
3	None (control)	37	537	391	72.8	146	27.2	Normal.

[a] Larvae not second-stage and, therefore, not infective; some were alive, but it is doubtful whether they could have subsequently become infective.

Examination of eggs from these cultures on the 37th day (Table 1) showed that some apparently normal and mature embryonated eggs were present in the cultures to which the chemicals had been added. The differences in the proportions of embryonated eggs in the treated and control cultures were of no significance. The embryos in most eggs in the culture treated with dichloropentanes exhibited marked structural abnormalities; to a far lesser degree chlorosol exerted the same effect.

Effect on development of nonembryonated eggs on moist soil.—In experiment

2 (Table 2), chlorosol prevented appreciable development of the eggs when applied to moist soil under conditions largely preventing the escape of its vapors from the culture dish for a period of about 4 days; under the same conditions dichloropentanes had little, if any, effect. A majority of the eggs which were in the process of development when the first examination of the cultures was made (Table 2) were probably alive, but it is doubtful whether those which were still not embryonated after 25 days were capable of further development. In any event, chlorosol evidently exerted a true lethal effect, rather than acting merely to retard development. Several hundred eggs isolated from culture 2 (Table 2) on the 16th day were washed and cultured in water; examination of the water culture 9 days later showed that only 1 egg had become embryonated. No others had undergone further development.

Experiment 3 was, therefore, performed to retest the effect of chlorosol and to determine (1) whether one or both of its constituents caused the lethal action, and (2) whether close confinement of the chemical vapors to the soil container was essential for effective action. The lethal effect of chlorosol on the eggs under conditions of close confinement of the vapors was confirmed by this experiment (Table 3). About half the eggs in culture 1 were evidently alive on the 13th day, since they underwent slight further development, but none became embryonated in 26 days. Apparently this result was due to the fact that less chlorosol was used than in the previous experiment. Since under the conditions of the experiment about 0.75 cc of ethylene dichloride apparently killed practically all of the eggs during the initial period of intensive exposure, and the same quantity of carbon tetrachloride was practically without lethal effect, the former compound was apparently the effective constituent of chlorosol. Neither ethylene dichloride nor chlorosol was effective in the quantities used unless the vapors were initially closely confined to the soil container.

Effect on embryonated eggs on soil.—Under conditions of close confinement of the vapors for 48 hours, 0.5 cc of chlorosol killed the larvae in all embryonated eggs on moist soil in a small dish in experiment 4 (Table 4); many eggs survived if the dish was merely covered throughout the test. Many eggs also survived similar treatment with dichloropentanes under both conditions; the time of exposure to this substance may be of more importance than the degree of confinement of the vapors.

As noted in table 4, the counts of living and dead eggs from cultures 2 and 4 are subject to qualifications. They correctly indicate that chlorosol affected the eggs more than dichloropentanes. But when the counts had been completed it was noted that the weight of the coverslip appeared to interfere with the response of the larvae to heat. Since this was not true of eggs from the control culture, nor when counts had been made 2 days earlier, the weakened condition of the larvae probably was responsible. The larvae in a majority of the eggs from culture 4 showed movement when the latter were transferred to water warmed to 37° C.; under the same conditions the larvae in a somewhat smaller proportion of eggs from culture 2 showed movement. These facts do not affect the validity of the above statement of the results of this experiment.

DISCUSSION

Chlorosol and ethylene dichloride were lethal to *Toxocara* eggs in experiments when used in proportions of about 32 to 65 cc per sq. ft. of soil surface. These experiments, of course, warrant no statement as to whether these chemicals could be effectively employed to kill ascarid eggs on the soil of kennel runways. They do definitely show the necessity for closely confining the chemical vapors to the soil for a period of 2 to 4 days, assuming that minimal quantities are to be employed,

TABLE 2.—*Effect of chorosol and dichloropentanes on the development of Toxocara eggs in moist soil in experiment 2*

Culture No.	Chemical used	Amount of chemical placed on soil surface	Interval in which lid of culture dish was sealed with petrolatum	Intervals from start of cultures to examinations of eggs	Number of eggs examined	Condition of eggs examined					
						Undeveloped and apparently dead		4-cell stage to tadpole stage		Embryonated	
		cc	*Hours*	*Days*		*No.*	*Per cent*	*No.*	*Per cent*	*No.*	*Per cent*
1	Dichloro-pentanes	1	96	16	543	214	39.4	79	14.5	250	46.1
				25	549	272	49.5	13	2.4	264	48.1
2	Chlorosol	1	96	16	529	526	99.4	3	0.6	0	0.0
				25	619	619	100.0	0	0.0	0	0.0
3	None (control)	None	96	16	521	153	29.4	98	18.8	270	51.8
				25	684	232	33.9	56	8.2	396	57.9

TABLE 3.—*Effect of chlorosol, ethylene dichloride, and carbon tetrachloride on development of Toxocara eggs on moist soil in experiment 3*

Culture No.	Chemical used	Amount of chemical placed on soil surface	Interval in which lid of culture dish was sealed with petrolatum	Intervals from start of cultures to examinations of eggs	Number of eggs examined	Condition of eggs examined					
						Undeveloped		6-cell stage to morula		Tadpole stage or fully embryonated	
		cc	*Hours*	*Days*		*No.*	*Per cent*	*No.*	*Per cent*	*No.*	*Per cent*
1	Chlorosol	0.75	48	13	556	551	99.1	5	0.9	0	0.0
				26	678	319	47.1	359[a]	52.9	0	0.0
2	do	0.75	(not sealed)	15	592	125	21.1	462	78.0	5	0.9
				26	735	152	20.7	269	36.6	314[b]	42.7
3	Carbon tetrachloride	0.75	48	15	537	91	16.9	164	30.5	282[c]	52.6
				26	706	113	16.0	118	16.7	475[b]	67.3
4	do	0.75	(not sealed)	15	581	65	11.2	64	11.0	452[d]	77.8
				26	673	85	12.6	51	7.6	537[d]	79.8
5	Ethylene dichloride	0.75	48	15	527	527	100.0	0	0.0	0	0.0
				26	595	593	99.7	2[e]	0.3	0	0.0
6	do	0.75	(not sealed)	15	593	145	24.4	447	75.4	1	0.2
				26	600	123	20.5	331	55.2	146	24.3
7	None (control)	.....	48	13	497	119	23.9	80	16.1	298[f]	60.0
				26	595	142	23.9	37	6.2	416[f]	69.9

[a] Nearly all in early cleavage stages.
[b] About half and half embryonated and tadpole stages.
[c] Nearly all in tadpole stage.
[d] Nearly all fully embryonated.
[e] One in 6-cell stage; one in 8-cell stage.
[f] All fully embryonated.

TABLE 4.—*Effect of 0.5 cc of chlorosol or dichloropentanes on vitality of larvae in embryonated eggs on moist soil in experiment 4*

Culture No.	Chemical added	Interval in which lid of culture dish was sealed with petrolatum	Interval from start of experiment to examination of eggs	Number of eggs examined	Condition of larva in eggs examined			
					Dead		Alive	
		Hours	*Days*		*Number*	*Per cent*	*Number*	*Per cent*
1	Chlorosol	90	7	402	402[a]	100.0	0	0.0
2	do	(not sealed)	9	236	148[b]	62.3	88[b]	37.7
3	Dichloropentanes	90	7	344	4	9.2	340	98.8
4	do	(not sealed)	9	157	94[b]	60.0	63[b]	40.0
5	None (control)	90	7	405	3	0.8	402	99.2
6	do	(not sealed)	9	305	3	1.0	302	99.0

[a] The larvae were very dark in color, vacuolated and appeared to have undergone obvious degeneration. Eggs from this culture were washed and transferred to water; examination 2 days later failed to reveal further development.

[b] For qualifications regarding this count, see text.

and hence suggest that practical difficulties would be encountered in finding an effective method of application. Any chemical used for the disinfection of kennel runs must be noninjurious to dogs or foxes. The above chemicals might satisfy this requirement particularly well because of their volatility.

The role of water solubility and of boiling point as determinants of the lethal action of the chemicals tested cannot be discussed on the basis of the limited data of these experiments. So far as ethylene dichloride is concerned, regarding it also as the effective constituent of chlorosol in these experiments, the data suggest that penetration into the eggs is favored on moist soil, where presumably only a thin film of water surrounds each egg and the chemical is mainly present as a vapor. Chitwood and Chitwood (1938, Jour. Parasitol., Sup. **24**: 34) state that substances in aqueous solution or emulsion do not have as effective a mode of dispersal in soil as do substances developing a high vapor pressure; they believe that cholesterol solvents developing a high vapor pressure act through their tendency to saturate the adherent moisture. Thereafter, these substances dissolve or penetrate the membranes of the organism. These authors found ethylene dichloride, among other organic compounds, effective against the nematode *Ditylenchus dipsaci* in soil.

Lucker and Shaffer (*loc. cit.*) found all of the substances mentioned in the present paper lethal to horse strongyle eggs in feces; they stated that dichloropentanes appeared to be the most effective and that its relative insolubility and high boiling point appeared to be advantageous. The writer now regards this statement as untenable. Owing to its low volatility, not all of the dichloropentanes were dispersed from the feces during the period of aeration and subsequent unpublished experiments with horse feces have shown that the continued action of this residue in the subsequent period of culture contributed to the lethal action observed. In the present experiments ethylene dichloride appeared to be more effective than dichloropentanes or carbon tetrachloride against *Toxocara* eggs; it is the most solu-

ble in water and has the lowest boiling point. Whether ethylene dichloride or chlorosol killed the eggs or merely delayed their development appeared to be conditioned, other factors being constant, by the concentration of their vapors. These experiments are not interpreted as indicating that carbon tetrachloride or dichloropentanes cannot kill *Toxocara* eggs; under the particular quantitative and other conditions of the experiments their action was not satisfactory.

SUMMARY

Some eggs of *Toxocara canis* became embryonated in cultures initially consisting of 1 part of chlorosol or dichloropentanes and 14 parts of water; chlorosol delayed the development of the eggs. Dichloropentanes prevented the larvae in embryonated eggs from reaching a normal structure to a greater extent than chlorosol.

Practically all eggs placed on moist soil in small dishes and exposed to small amounts of chlorosol or ethylene dichloride, under conditions minimizing loss of the chemical vapors from the containers in an initial period of 48 to 96 hours, failed to become embryonated or undergo appreciable development and were evidently killed. Practically all embryonated eggs similarly exposed to chlorosol for 96 hours were also killed. Under the same conditions, dichloropentanes and carbon tetrachloride were ineffective in preventing embryonation of the eggs; development was delayed by carbon tetrachloride. Dichloropentanes was not effective against embryonated eggs under similar conditions.

Since minimal quantities of ethylene dichloride, regarded also as the lethal constituent of chlorosol in the experiments, were not effective against the eggs unless the vapors were closely confined to the soil for from 2 to 4 days, difficulties are to be anticipated in finding a method for effectively applying the chemical to soil under practical conditions.

Studies on oxyuriasis. XXI. The chemistry of the membranes of the pinworm egg.[1] Leon Jacobs and Myrna F. Jones, National Institute of Health, U. S. Public Health Service, Washington, D. C.

During the summer of 1937, one of us (L. J.) was engaged for a short time in collaboration with Dr. B. G. Chitwood in studying the chemical constitution of various nemic skeletoids. The present note is the outcome of work with the eggs of *Enterobius vermicularis* along these lines with the purpose of obtaining from a study of the egg membranes some information which might lead to the selection of an effective ovicide. In 1938, Chitwood (1938) published the results of his investigations which included chemical studies on the egg membranes of *Ascaris lumbricoides* and *Dioctophyme renale*. His conclusions as regards *Ascaris* are in substantial agreement with those of Wottge (1937), who made a study of the chemistry of the egg membranes and of their method of formation. Both authors describe 3 membranes, viz.: An outer proteinaceous membrane; the shell proper, which is composed of chitin; and an inner "fibrous" lipoidal membrane. The latter two are secreted by the egg cell after fertilization. There is a difference of opinion concerning the origin of the outermost membrane, Chitwood believing that it is formed as a secretion product of the uterus, and Wottge expressing the opinion that it originally exists as a sort of "ectoplasm" of the egg and is raised away from the egg by the secretion of the other two membranes. On this point, Zawadowsky and Schalimov (1929) agree with Wottge, stating that all the membranes are formed by the undivided egg.

[1] The authors wish to thank Mrs. Paula Weeks Taylor for assistance in some of the tests in the early part of this work.

For a bibliography of the work on nematode eggs, the reader is referred to the extensive bibliographies in the papers of Chitwood and Wottge. The only work on the chemistry of pinworm eggs known to the authors is that of Zawadowsky and Schalimov (1929), who described 4 membranes on the basis of observations of the interfaces formed during the penetration of cedar oil into the egg. They did not make a chemical study of all the membranes but tested the innermost one and stated that it is lipoidal in nature and also that it undergoes a "maturation" after the embryo has reached the tadpole stage. We have been able to demonstrate only 3 membranes chemically and, since all our observations were made on eggs developed beyond the tadpole stage, no comment can be made here as to the "maturation" of the lipoidal membrane.

The outermost membrane of the pinworm egg, corresponding to the "albuminous" membrane of the *Ascaris* egg, swells and dissolves when the egg is placed in gastric and pancreatic juices and in sodium hypochlorite. It swells but does not dissolve when the egg is placed in dilute acetic acid (1 to 3 per cent), 1 per cent HCl, and 1 per cent KOH. It swells and takes an orange color when subjected to the vapors of fuming nitric acid and ammonium hydroxide successively, thus indicating that it is a protein. It is insoluble in water and is therefore not an albumin, as its name implies. On the basis of his findings, Chitwood concludes that the "albuminous" membrane of the *Ascaris* egg is "certainly not an albumin, collagen, fibroid, or keratin. . . . One would presume it to belong to the conjugated proteins, such as mucoids, which form a similar covering of the egg in other animals." On the other hand, Wottge concludes on the basis of staining with Sudan III that some lipoidal material is also present in this membrane. Because the membrane of the pinworm egg merely swelled without dissolution when placed in dilute acids and alkalies in our experiments, attempts were made to dissolve the swollen membrane by placing it in salivary juice and in lipoid solvents. These attempts were unsuccessful. The nature of the protein composing the outermost membrane is therefore still in question.

The shell proper lies between this proteinaceous membrane and the innermost lipoidal membrane. It was tested for chitin by the methods outlined by Campbell (1929). The eggs were heated in saturated KOH at 160° C. for 15 minutes in a small sealed tube immersed in a glycerine bath. After such treatment, the shell proper was the only material left undissolved. It stained brown in iodine-potassium-iodide solution and turned violet on the addition of dilute sulphuric acid. Dilute acetic acid dissolved the shell after the KOH treatment. After having been previously stained with iodine-potassium-iodide, the treated shell lost its color on going into solution in concentrated sulphuric acid. The only organic substances known to withstand superheating in concentrated KOH are chitin and cellulose; cellulose in this case is eliminated from consideration because it is insoluble in dilute acetic acid and does not stain in iodine-dilute-acid, and because of the manner in which it dissolves in concentrated sulphuric acid, *i.e.*, swelling and turning blue. It is concluded, therefore, that the shell proper is composed of chitin.

On one occasion when the shell was properly orientated after heating the egg with KOH, it was observed that the insoluble shell was lacking at the hatching area. On the basis of this and other observations, it is considered possible that only the outer membrane and the lipoidal membrane separate the embryo from the outside at this particular region of the egg. Cobb (1890) refers to this hatching area as a porous region and figures small pores running through the shell. The present writers have never been able to observe any such structures in this minute area. However, it has been continually noted that the shell membranes are thinner at this point, which observation may be explained by the possible lack of chitin at the hatching area.

In attempting to dissolve away the shell proper with sodium hypochlorite in order to test the lipoidal membrane of the egg, it was noticed that an outer layer of the chitin first split away from the rest of the shell, the split beginning on both sides of the hatching area (but not above it—an additional indication that chitin is lacking there) and continuing back until the entire layer became detached from the rest of the shell. After this split had occurred, part of the shell proper still remained enclosing the lipoidal membrane and the embryo; this remaining part dissolved more slowly in the hypochlorite solution. This layering of the chitin shell is probably the reason Zawadowsky and Schalimov saw 4 different interfaces during the penetration of cedar oil into the egg and concluded that there were 4 distinct membranes.

The innermost lipoidal membrane was tested both directly and indirectly in order to ascertain its nature more specifically. Indirect tests were necessary at first, because the membrane is so delicate that it could not be tested alone. A test was devised which took advantage of the impermeability of this membrane to the vital stain, neutral red. Eggs from which the outermost membrane had been digested by artificial gastric juice leaving only the shell and the lipoidal membrane were washed in water and observed under the microscope in a solution of neutral red. Even over a period of several hours, no penetration of the dye into the egg could be observed. The neutral red was then drawn off, the egg was dried, and neutral red was again added to determine whether or not drying changed the permeability of the lipoidal membrane. The dye still did not penetrate into the egg. After this fact was ascertained, the neutral red was again drawn off, the egg was dried and washed with various fat solvents. After such treatment with alcohol, acetone, ether, benzol, and chloroform, when neutral red was again added to the slide, the embryo inside the egg assumed a deep red color. This was taken to indicate that these fat solvents had dissolved the lipoidal membrane; it could not be attributed to the simultaneous dissolution of a thermolabile sterol membrane around the embryo (Chitwood, 1938, p. 74), because hatched larvae, which had never been subjected to the action of fat solvents, became stained with the dye.

By using eggs which had been dried before treatment with gastric juice, it was later possible to obtain larvae which were enclosed only by the lipoidal membrane, the shell proper having become dislodged from the embryo after the digestion of the outer membrane. This separation again indicates the lack of chitin at the hatching area, because the embryo remained enclosed in the lipoidal membrane, which it always ruptures on hatching, and did not push its way out of the shell, and yet the shell became dislodged. Apparently the embryo enclosed in the lipoidal membrane was shaken out of the shell through the hatching area during the handling of the dish containing the eggs. These eggs were easier to test, and the lipoidal membrane was able to withstand without rupturing the drying which was necessary before it could be subjected to the action of the fat solvents. During direct observation on these eggs, the lipoidal membrane was seen to dissolve in all of the above-mentioned fat solvents and in xylol. The membrane was seen to disappear entirely and the embryo became free. The membrane assumed no stain on the addition of Sudan III and Nile blue sulphate. On the basis of these tests, it is concluded that this innermost membrane is a sterol.

As stated by Zawadowsky and Schalimov, this sterol membrane is an effective barrier against the penetration of lethal agents into the egg. The larva enclosed in it remains undamaged and active even after the outer protein membrane has been dissolved away or swollen by alkalies and acids. If, however, the sterol membrane is dissolved, the embryo is immediately affected by these reagents. The proteinaceous membrane also appears to have a protective role against chemical agents; it is impossible to dissolve the lipoidal membrane without first removing the protein

covering of the egg, because fat solvents cannot penetrate through it. On the other hand, our observations and those of Zawadowsky and Schalimov and of Chitwood indicate that the chitin shell serves only for mechanical support and protection.

On the basis of these findings it appears that an effective ovicide must contain substances which will dissolve proteins and lipoids in order to reach the embryo.

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Some factors governing the success of chemical treatment of soil for nematode control. GERALD THORNE, U. S. Bureau of Plant Industry, Salt Lake City, Utah.

During the years 1923 to 1926 a number of chemical control experiments for the sugar-beet nematode, *Heterodera schachtii*, were carried on at the Salt Lake City, Utah station, of the U. S. Bureau of Plant Industry. These control measures were unsatisfactory and for that reason the results were not published except in a general statement by the writer (U. S. Dept. Agr. Farmers' Bull. 1514). In these experiments certain fundamental aspects were considered which generally have been overlooked by other investigators in the chemical treatment of soil in the field, viz., distribution of the nematode population, soil structure, soil moisture content and actual penetration of the chemical applied. That the problem of chemical control of nematodes in the field may be placed on a more comprehensive basis, complete data on one of the most successful of these treatments are here presented.

Soil structure.—The field selected in this case is a medium sandy loam typical of a large portion of the irrigated soils of the western states. A profile taken in 2-inch sections (Fig. 1, A) first shows about 12 inches of loose cultivated soil with a high organic content, largely derived from frequent applications of barnyard manure.

Immediately below this tilled portion is the "plow-sole," a compact top layer of the subsoil which has been mechanically packed by the powerful wedging action of plows and the weight of tractors or horses pulling them. In addition it is made still more compact and impervious by the accumulation of minute soil particles and colloids carried down from the tilled portion by percolating water and up from the subsoil by capillary forces. This "plow-sole" is present in practically all cultivated soils of the western states and constitutes a formidable barrier to the penetration of gases and solutions of chemicals used in soil treatments.

Below this "plow-sole" lies the subsoil, a more or less calcareous, cemented structure with an unbroken profile except for numerous channels formed by deep-rooted plants such as alfalfa. Penetration into this subsoil is necessarily a difficult matter and the nematodes inhabiting it have an excellent opportunity to escape any method of soil treatment.

Nemic distribution.—Population figures are based on 6 one-millionth acre*

* 1×10^{-6} acre = 6.27264 in.^{2}

columns of soil taken to a depth of 24 inches in heavy galvanized iron tubes.* Each column was removed in 2-inch sections, the corresponding sections of 6 lots were then carefully mixed and $\frac{1}{6}$ of the total mass weighed out. The brown cysts of *Heterodera schachtii* were segregated by the Cobb sifting and gravity methods (Estimating the nema population of soil with special reference to the sugar-beet and root-gall nemas. U. S. Dept. Agr., Bur. Plant Indus., Off. Agr. Tech. Circ. 1, 1918). Each cyst was opened and the eggs examined for living larvae which usually were easily distinguished from the dead ones by the fact that when the eggshell was broken they instantly straightened out while those which were dead

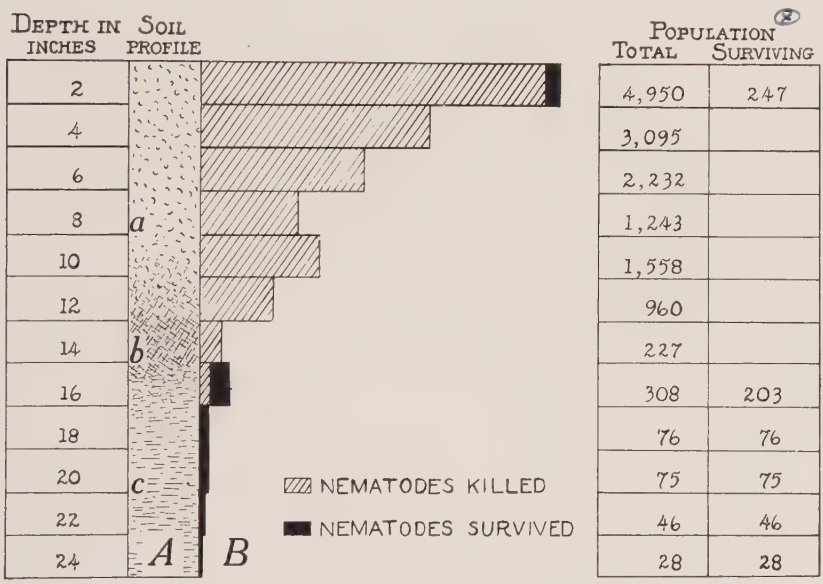

FIG. 1. Diagram showing soil profile, distribution of *Heterodera schachtii* and kill of *H. schachtii* by application of calcium cyanide at the rate of 800 pounds per acre. A—Soil profile; *a*, tilled portion; *b*, plow-sole; *c*, subsoil. B—Relative portions of the *H. schachtii* population in each two inches of soil.

remained partially folded as they had been in the egg. Because of the small number of cysts present from 13 through 24 inches these 6 2-inch sections of soil were not subdivided but examined entire.

At the time of treatment almost $\frac{1}{3}$ of the *Heterodera schachtii* population was in the top 2 inches of soil (Fig. 1, B). Concentration to this extent at this level was somewhat higher than usual and was brought about by the plow's bringing soil to the surface from lower levels where beet roots had been numerous and great numbers of nematodes had developed the previous year. There was a fairly constant decrease of the population down to the 16-inch level. Below this the cysts were scarce and small in size, resulting in unusually low populations for these depths.

Moisture.—At the time of treatment the water content of the soil was very uniform, varying from 14 per cent near the surface to 16 per cent at 24 inches. This was just about ideal from the standpoint of keeping all nematodes active and therefore more susceptible to chemical treatment.

Temperature.—The midday temperature at the surface was 21° C., from which it decreased rather uniformly to 16° C. at 24 inches. Combined with the favorable moisture content these temperatures made excellent conditions.

Application of chemical.—Granular calcium cyanide, $Ca(CN)_2$, was applied at

* Effective diam. = 2·82" (approx.).

the rate of 800 pounds per acre. This was done with the aid of a special applicator attached to the plow beam which dropped the cyanide into the furrow just ahead of the plow. Plowing depth was from 10 to 12 inches and most of the cyanide was placed at that level. After plowing, the field was harrowed down to a fine mulch and levelled. By the time this was completed the cyanide gas rising from the soil was so strong that it was almost impossible to continue work.

Effectiveness of treatment.—Diffusion of the gas at the surface enabled about 5 per cent of the *Heterodera schachtii* to escape in the top 2 inches. On small areas this might be prevented by a tar-paper cover as suggested by Godfrey *et al.* (1934, Phytopath. **24**(12): 1332–1346), but this method is impractical under ordinary field conditions. In the stratum of 3 through 14 inches apparently all of the nematodes were killed while in that from 15 through 24 inches the greater portion was uninjured. Doubtless a few nematodes were present below the 24-inch level but this soil was not sampled.

The total per acre population of 14,798,000,000 *Heterodera schachtii* was not an unusually high number for a severely infested field. While the surviving population of 576,000,000 was only 4.56 per cent of the total it was, nevertheless, ample to reestablish the pest in dangerous numbers in one season when beets were immediately grown on the field.

The calcium cyanide was applied April 8, 1924. On April 21 the above-discussed soil samples were taken and beets were planted in the usual manner. Subsequently the young beets showed some injury from cyanide residues but made a fairly normal growth during the season. On September 10 five beets were dug and, together with the immediately surrounding soil, examined for *Heterodera schachtii*. An average of 176 females was present on each beet and the following year when beets were again planted the plot was severely infested.

Discussion.—The above detailed studies of the distribution of *Heterodera schachtii* in the soil and the kill made by granular calcium cyanide demonstrate why such powerful poisons have failed to give complete control even when applied in amounts which are far too expensive for general use. Penetration by gases through the plow-sole and underlying subsoil is very difficult, yet an important portion of the nematode population is present in these areas. Later applications of $Ca(CN)_2$ were made at a depth of 14 to 16 inches, using a subsoil plow attachment but a large number of the nematodes still escaped in the hard, unbroken soil.

Heterodera schachtii is an unusually favorable species upon which to make population studies for in the spring all of the larvae are in the eggs within the brown cysts and these are easily separated from the soil. Free-living species are always present in any cultivated soil and these may be used as indicators of what a chemical treatment has accomplished for if dorylaims, mononchs and cephalobs are still alive after treatment it is certain that the more resistant tylenchs such as *H. schachtii*, *H. marioni*, *Ditylenchus dipsaci* and *Pratylenchus pratensis* have also escaped, especially those larvae and eggs protected by pieces of roots in which they remain embedded.

Efficient spacing of soil fumigants for field applications. A. L. TAYLOR, U. S. Bureau of Plant Industry, Tifton, Ga.

When liquid soil fumigants, such as chloropicrin and carbon bisulphide, are applied to the soil, they evaporate to form gases which diffuse and extend their lethal action to certain distances from the point of application. This distance depends on several factors, among which are the physical and toxic properties of the fumigant, the amount applied, the organism to be controlled, the soil type, its physical condition, temperature and moisture content. Practically, the distance for any given set of conditions can only be found by experiment. The reader is referred

to Newton, Bosher and Hastings (1937, Canad. Jour. Research, sect. C, **15**(5): 182–186), Miller and Allison (1935, Soil Sci. **40**(2): 173–178) and Chitwood (1939, Proc. Helminth. Soc. Wash., this issue) for experimental methods.

Once the maximum distance of action of the chemical has been found, the problem becomes that of spacing the application points in the plot to be fumigated so that the most efficient use is made of the fumigant, *i.e.*, so that no point in the plot is more than the calculated distance from a point of application and furthermore, so that the areas treated by the various applications overlap a minimum amount.

Where the soil is homogeneous, the fumigating gases will diffuse evenly in all directions from the point of application, fumigating an area circular in relation to the soil surface. The radius of this circular area will be a distance equal to the maximum effective "range" of the fumigant. For convenience, this distance will be designated as k, and defined as the maximum distance from the point of application of the fumigant to the point where it produces the desired results, which is usually the point where all the parasites in the soil are destroyed.

As each application will fumigate a circular area of soil with radius k and diameter $2k$, circles of this diameter must be fitted in the plot in such a manner as to entirely cover it with a minimum of overlapping. This requirement is met when the centers of the circles are placed at the corners of equilateral triangles of such size that their centers are at a distance k from each corner. Figure 1, A,

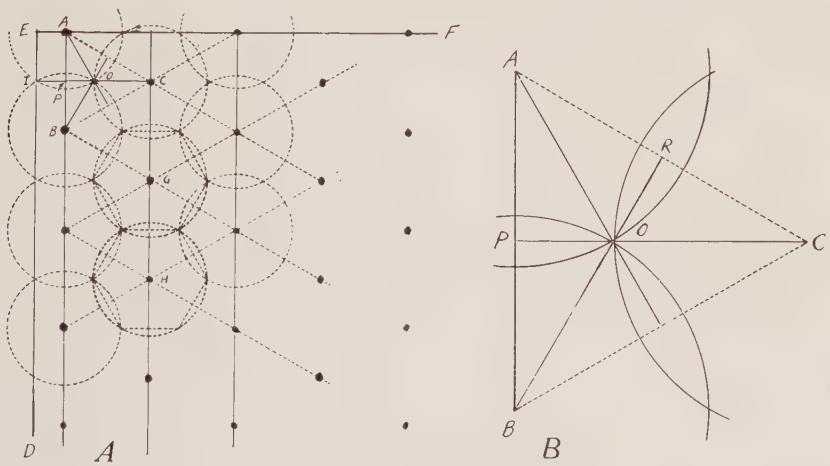

FIG. 1. Spacing of soil fumigants.

shows the arrangement of the circles in the plot, the corner of which is represented by the line DEF. Rows of application points run from top to bottom of the figure (solid lines). Circles represent the area fumigated by each application. Dotted lines complete the triangles.

Figure 1, B, is the triangle ABC from figure 1, A. As the triangle is equilateral, AO, BO and CO bisect the three angles, are perpendicular to the opposite sides and are of equal length. AO is the hypotenuse of a right triangle AOP with one 30° angle, and its length is k. The cosine of 30° is 0.866, so the length of AP is $0.866k$. Then the length of AB is twice that amount or $1.732k$, the distance between application points in the row. The sine of 30° is 0.500 and the length of line PO is $0.5k$. Since OC is equal to k, the length of PC is $1.5k$, the distance between rows in the plot.

As can be seen from figure 1, A, the soil will be fumigated to a distance IP to

the left of the first row of application points. IP is equal to OP or to $0.5k$. The first row of application points should be this distance from the edge of the plot.

Figure 1, B, also shows that the first application point in the second row should be at a distance equal to AP or $0.866k$ from the edge of the plot, where the corners of the plot are square.

Since the circles overlap, each application adds a hexagonal area to the fumigated soil. The area of a hexagon is given by the formula $2.598s^2$, where s is one side of the hexagon. As the sides of a hexagon are equal to the radius of a circumscribed circle, the sides of the hexagon are of length k, and its area is $2.598k^2$. The same result is obtained by multiplying the distance between application points by the distance between rows.

The number of application points in a given area can be obtained by dividing the number of square units in the area by $2.598k^2$. From this figure and the amount of fumigant per application, the total amount of fumigant required for any given area can be easily calculated. Where k is expressed in inches, the number of applications per acre will be 2,414,411 divided by k^2. The number of application points per square yard will be 498.8 divided by k^2, and the number of application points per square foot will be 55.427 divided by k^2.

Example: It is found by experiment that 1 cc application of a fumigant will kill all the plant parasites within 6 inches under given conditions. There are 273 cc of the fumigant per pound.

Then:

The value of k is 6 inches.

Application points will be 1.732 times 6 inches apart in the rows, or 10.4 inches (10.392 inches exactly).

Rows of application points will be 1.5 times 6 inches apart, or 9 inches.

The first row of application points will be 0.5 times 6 inches, or 3 inches, from the edge of the plot.

The first application point in the second and all the other even numbered rows will be 0.866 times 6 inches, or 5.2 inches (5.196 inches exactly), from the edge of the plot.

The number of application points per acre will be 2,414,411 divided by 6^2 or 67,067. The number of application points per square yard will be 13.85. Since each application point takes 1 cc, 67,067 cc or 245.6 pounds will be required for 1 acre.

The first three columns of the table show spacing of rows of injection points and the spacing of these points in the rows. The fourth column gives the number of injection points per square foot. The remainder of the table gives the number of cubic centimeters required for a square foot for various combinations of k values and amount per injection. Once the amount per square foot is obtained from the table the amount needed for any given area is quickly computed.

Soil fumigations are generally expensive and the labor required is a comparatively small portion of their cost, but it should also be considered. Other things being equal, labor will be in proportion to the number of application points in a given area. The number of application points in any area varies inversely as the square of the k value. If the amount of fumigant per application is multiplied by a certain factor, the k value of the resulting amount should be the square root of that factor times the original k value. If it is less than this amount, it indicates a loss in efficiency, for a larger quantity of fumigant will be needed for a given area. If it is equal to or more than this amount, it represents a gain in efficiency, for fewer application points and less labor will be required.

Example: With a certain fumigant, 10 cc gives a k value of 6 inches. Under

TABLE 1.—*Soil fumigants—spacing and amounts*

K values	Distance between		Injection points per square foot	Cubic centimeters per injection											
	Rows of injection points	Injection points in row		1	2	3	4	5	6	7	8	9	10	15	25
				Cubic centimeters per square foot											
Inches															
4	6.0	6.9	3.46	3.46	6.92	10.38	13.84	17.30	20.76	24.22	27.68	31.14	34.6	51.9	86.5
5	7.5	8.7	2.21	2.21	4.42	6.63	8.84	11.05	13.26	15.47	17.68	19.89	22.1	33.1	55.2
6	9.0	10.4	1.53	1.53	3.06	4.59	6.12	7.65	9.18	10.71	12.24	13.77	15.3	22.9	38.2
7	10.5	12.1	1.13	1.13	2.26	3.39	4.52	5.65	6.78	7.91	9.04	10.17	11.3	16.9	28.2
8	12.0	13.9	0.866	0.87	1.73	2.59	3.46	4.33	5.19	6.06	6.92	7.79	8.6	13.0	21.6
9	13.5	15.6	0.684	0.68	1.36	2.05	2.73	3.42	4.10	4.78	5.47	6.15	6.8	10.2	17.1
10	15.0	17.3	0.554	0.55	1.10	1.66	2.21	2.77	3.32	3.87	4.43	4.98	5.5	8.3	13.8
12	18.0	20.8	0.384	0.38	0.76	1.15	1.53	1.92	2.30	2.68	3.07	3.45	3.8	5.7	9.6
14	21.0	24.2	0.282	0.28	0.56	0.84	1.12	1.41	1.69	1.97	2.25	2.53	2.8	4.2	7.0

the same conditions, 20 cc gives a k value of 8 inches. Which is the most efficient amount?

Since the amount is multiplied by a factor of 2, the k value should be multiplied by the square root of 2 or 1.41 for equal efficiency. Six inches times 1.41 equals 8.46 inches. Since the k value obtained by experiment is only 8 inches, the larger amount is the less efficient. The 10 cc amount would require 13.85 applications per square yard with 138.5 cc of fumigant. The 20 cc amount would require 7.79 applications per square yard with 155.8 cc of fumigant.

SUMMARY

If k is the maximum distance from the point of application of a soil fumigant to the point where it produces the desired results, the most efficient utilization of the fumigant is obtained when:

Application points are located in straight parallel rows $1.5k$ apart.

Application points are $1.732k$ apart in the rows.

The first application point in the first and other odd-numbered rows is located on a line perpendicular to the rows, and the first application point in the second and other even-numbered rows is made at a distance $0.866k$ from this line.

Each application under the above condition will fumigate an area $2.598k^2$.

Where k is expressed in inches, there will be 2,414,411 divided by k^2 application points per acre, and there will be 498.8 divided by k^2 application points per square yard, and 55,427 divided by k^2 application points per square foot.

From the number of applications required for a given area, the number of units per application and the units per pound or kilogram of the fumigant, the total weight required for the area can be easily calculated.

A rapid method for determining k values of nematocides. B. G. CHITWOOD, U. S. Bureau of Plant Industry, Babylon, N. Y.

Recently carbon disulphide and chloropicrin have been applied as soil nematocides with variable though promising results by several workers. The use of such volatile substances by point injection (Johnson and Godfrey, 1932, Indus. & Engin. Chem. **24**: 311–318; Taylor, 1939, Proc. Helminth. Soc. Wash., this issue) introduces several new problems that are not so important when chemicals are applied broadcast. The chief problem is the determination of the maximum horizontal and vertical radii of dispersion at which a given dose of the nematocide effectively acts upon the nemas under a given set of conditions.

Field tests using the empirical method for selection of dosage rates and spacing of injections have been conducted on a moderate scale by several workers (Johnson and Godfrey, *loc. cit.;* Neller and Allison, 1935, Soil Sci. **40**(2): 173–178). These tests have been supplemented by pot tests (Godfrey, Oliveira and Hoshino, 1934, Phytopath. **24**(12): 1332–1346). Most of this work has been done with *Heterodera marioni,* the root-knot nematode. While sterilization of field and greenhouse soils is the ultimate goal of any work on soil nematocides, this is expensive, slow, and not necessarily the best procedure for preliminary work.

I-Type Tests.—Newton, Hastings and Bosher (1937, Canad. Jour. Research, Sect. C, **15**: 182–186) have originated an experimental technic for determining the efficacy of dosage rates at varied distances from the point of injection. These authors placed masses of *Ditylenchus dipsaci,* the bulb or stem nematode, in the form of nema wool in small vials which were inserted in a cardboard strip at intervals of 2 inches. They then buried the cardboard in soil at a depth of 6 inches and injected doses of 1, 2, 4 and 6 cc of chloropicrin at one end. We shall term this the I-type test. The vials were recovered after an exposure of 1 week and the

nemas were examined. On this basis the authors concluded that 1 cc of chloropicrin killed all the nemas at a distance of 6 inches while 2, 4, and 6 cc did not show a complete kill at 8 inches. Taylor (1939, *loc. cit.*) has defined *k* as the maximum distance from point of application at which a nematocide kills all noxious soil nemas. Our first problem is how to determine *k* values under varied conditions with constant dosage rates, and under constant conditions with varied dosage rates. One would naturally suppose the *k* value of 1, 2, 4, and 6 cc of chloropicrin to be 6 under the conditions tested by Newton, Hastings and Bosher. The writer has carried out a large number of such tests using *Ditylenchus dipsaci*. The results have been extremely interesting and the I-type test appears to be a highly recommendable procedure for testing the relative efficacies of nematocides. It places all the reagents on an equal basis and a large number of tests can be carried out in a single day. The results are quickly obtainable and the environmental factors influencing nematocidal efficacy can be segregated. However, it puts the nematocide at a disadvantage since in field or greenhouse tests the doses from several points of injection are equal distances apart and all may contribute to the death of nemas at the intersection of their dispersion radii. All compounds must be volatile to a greater or lesser extent if they are to be effective when applied by the injection method. Each adjacent dose would add to the vapor pressure of the reagent at any point between adjacent points of injection. This factor we shall term the *summation effect* and it should be most noticeable at the intersection of the radii.

X-Type Tests.—In order to measure a possible summation effect the writer designed a test using 2 crossed cardboard strips in the form of an X with a vial at the intersection and vials at 2-inch intervals from the points of injection. Equal injections were made at the end of each of the 4 projecting arms of the cardboard strips. A kill at the intersection does not indicate the limit of the killing range and before this can be determined one must make several X-type tests wherein the arms of the X differ in length. The record of 9 X-type tests duplicated by 9 I-type tests will serve to demonstrate summation effect. Three different doses of chloropicrin were applied at different times as indicated in table 1. To avoid, in this paper, a discussion of environmental conditions, data on this point are not presented.

TABLE 1.—*Comparison of chloropicrin k values determined by I-type tests with those determined by X-type tests*

	Set 1			Set 2			Set 3			Total	Average
Dosage in cc	1	2	3	1	2	3	1	2	3		
k values, I-type tests	2	4	4	6	6	4[a]	2	6	6	40	4
k values, X-type tests	6	6	6	6	8	10	6	8	10	68	7
Differences in *k* values	4	2	2	0	2	6	4	2	4	28	3

[a] Note variation. This is common in I-type tests but less marked in X-type tests.

Vertical Tests.—Facts recently presented by Thorne (1939, Proc. Helminth. Soc. Wash., this issue) led us to realize that horizontal tests are not an entirely adequate index to the killing range of a nematocide. Theoretically one might presume that the radius of vertical kill would be identical with the horizontal so that the killing range might be represented as a sphere. But experience has led the writer to doubt that vertical and horizontal *k* values are identical. Hence an addition was made to the X-type test. This addition consists of a vertical cardbroad strip carrying vials of nemas with the top vial just under the soil surface and vials at 2-inch intervals. The vertical strip is placed next to the intersection of the X (Fig. 1, C and D). In practise we first had strips extending only upward.

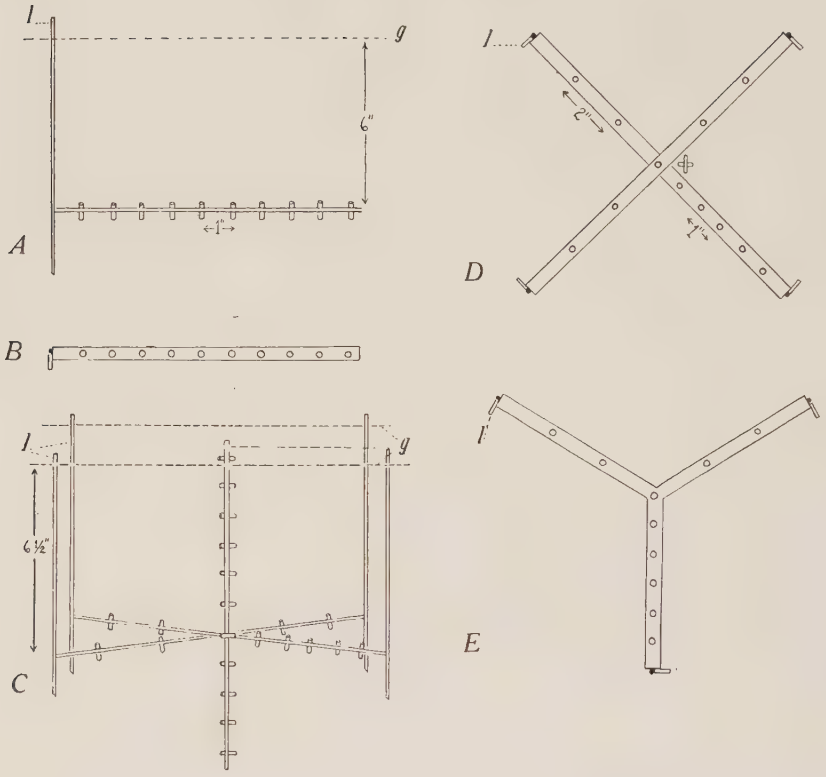

FIG. 1. Various types of nematocide tests. A—I-type test; profile. B—I-type test; subsurface view from above. C—X-type test with vertical arm; profile. D—X-type test with vertical arm; subsurface view from above. E—Y-type test; subsurface view from above. *g*, ground level; *l*, garden label.

Later the strips were extended below the horizontal X. The importance of vertical tests was readily demonstrated in a series of 7 modified X-type tests with the horizontal strips buried 8½ inches, with no soil cover and with no rainfall during the experimental period. In these 7 tests a different dosage of chloropicrin was used with each, *i.e.*, 1, 2, 3, 4, 5, 6, and 10 cc per injection, and the injections were made 6, 8, 10, 12, 12, 12, and 14 inches respectively from the point of intersection. A complete kill was obtained in the top vial in only 3 tests (5, 6, and 10 cc doses) though in all cases the vials at the intersection and all vials along the horizontal X showed a complete kill.

In another set consisting of 9 tests 3 had no soil cover, 3 had a glued paper cover and 3 were sprinkled with water after injection. For each type, injections were 6 inches, 8 inches, and 10 inches from the point of intersection and the corresponding dosages were 1, 2, and 3 cc respectively. In all instances the depth of injection was 6½ inches. In 2 cases living nemas were recovered from the top vial of the vertical series, one case being the 3 cc dosage test (injected 10 inches from intersection) with glued paper cover and the other 3 cc dosage test (injected 10 inches from intersection) without cover. In the latter case, however, the upper vial was above soil at the end of the test period. In no case, on the basis of horizontal tests, should the nemas in the top vial have been killed. The nematocide apparently tends to go upward. Under particular conditions some kind of cover would be

indicated. In the selection of a cover the condition of the soil would need to be considered.

Y-Type Tests.—When injections are placed in staggered rows, as described by Taylor (*loc. cit.*), nemas located at the intersection of the circles representing presumptive radii of dispersion, are exposed to the doses from 3 adjacent points of injection. Lines drawn from each of these points of injection to the point of intersection result in the formation of a Y. To obtain k values comparable to field tests, vials may be placed at intervals along Y-shaped cardboard strips of different sizes. An equal injection is then made at each of the three ends of the Y. Such tests are now in progress and will probably supplant the X-type test. Vertical strips should be added at the intersection just as for the X-type test.

In order to obtain a comparison of k values obtained by X-, Y-, and I-type tests the records of 12 X-type and 12 Y-type tests duplicated by 6 I-type tests are given in table 2. All of these were done at the same time but there were 3 sets of 10 tests, each set being under different conditions. Set 1 was covered by glued paper, set 2 was without cover, and set 3 was sprinkled with water. Since a light rain occurred during the experimental period, set 2 might be considered as having been sprinkled once and set 3 sprinkled twice. In the table the number of inches to the intersection of Y- and X-type tests is designated by the number subscribed after the letter indicating the type of test. When spaced beyond the apparent limit of this dosage (6 inches) it will be seen that records become erratic and a Y- or X-type test may give no higher k value than an I-type test.

TABLE 2.—*Comparison of 1 cc chloropicrin k values determined by I-type tests compared with those determined by Y-type and X-type tests*

Test	Y_4	X_4	Y_6	X_6	Y_8	X_8	Y_{10}	X_{10}	I_{10}	I_{10}	Total	Average
Set 1	4	4	6	6	5	5	4	4	4	4	46	4.6
Set 2	4	4	6	6	5	8	4	10	5	5	57	5.7
Set 3	4	4	6	6	8	8	8	8	5	5	62	6.2
Total	12	12	18	18	18	21	16	22	14	14		
Average	4	4	6	6	6	7	5	7	5	5		

Miscellaneous points.—In making any of the tests caution should be exerted to avoid interference from adjoining tests. Recently we have refined our technic by inserting vials at 1-inch intervals along one limb of the X or Y and at the same intervals along the vertical strip. Examination of the vials closest to the injection point is often unnecessary.

Material is likely to fall out of the vertical vials. This may be prevented by covering the end with cheesecloth after filling the vial with soil. Migration of the nemas from the vials is not ordinarily an important factor but during wet weather it might occur to such an extent that only dead nemas would remain within the vials. Covers of bolting silk will prevent migration but may lower the k value determinations to a minor extent.

The best time interval for a test probably depends upon temperature as incomplete results seem to indicate that where a week is sufficient with a high soil temperature, 10 to 12 days is preferable with a low soil temperature. Soil type, water holding capacity, and percentage of saturation, as well as soil temperature, should be recorded in order that the information may be applied. These data were purposely omitted from the present paper to prevent possible misinterpretation due to the lack of an extended discussion. All information is relative.

SUMMARY

Technics for determining the killing range of a nematocide are discussed. The I-type test (horizontal) is considered of value in selecting chemicals and determining dosage rates but not for the determination of row and hole spacing in plot or field. After selecting a few promising dosage rates, X- or Y-type tests should be performed using various arm lengths and including a vertical arm. Information so obtained should be applied only under conditions similar to those of the test.

Frames for spacing injections of soil nematocides. B. G. Chitwood, U. S. Bureau of Plant Industry, Babylon, N. Y.

The injection of experimental plots and greenhouse beds with nematocides according to the plan presented by Taylor (1939, Proc. Helminth. Soc. Wash., this issue) presents a problem in the accurate spacing of injection-holes. If nematocides are applied in the most economical manner, the rows and holes must be placed as far apart as possible, consistent with k value determinations. Slight errors in spacing may easily leave untreated sectors which would permit reinfestation and thereby defeat the purpose of the injection.

With this possibility of error in mind several types of frames have been designed, based on Taylor's formulae. Undoubtedly many more patterns could be made, some of which might show slight economies in application for particular beds. The use of some type of frame provides for accuracy of spacing and does not materially increase the labor. If one considers the time necessary for accurately spotting the points to be injected, it saves considerable time.

Three types of frames applicable to greenhouse beds of various sizes will be described. Variants of these will cover practically any commercial bed. All frames are made of a rectangular piece of galvanized iron or 3-ply fir panel nailed to a narrow wooden sash. For convenience we shall assume in all instances that previous tests have shown that an injection of 2 cc of nematocide has a k value of 6 under the chemical and structural condition of the particular soil, the soil moisture, and the temperature. We shall further assume, for orientation in description, that the writer is standing in the greenhouse bed facing the end of the bed. In describing the construction and operation of frames the same position is maintained but for the frames the long side usually corresponds to the width of the bed.

Frame I. Designed for beds 45.6 to 48.5 inches wide.—As an example, let us assume that we have a coldframe or greenhouse bed 4 feet wide by 40 feet long. With $\frac{3}{4}$-inch side and end boards the bed would have an inside measurement of 46.5 by 478.5 inches. Using the customary method of injection by transverse rows, the frame would be $3k$ or 18 inches wide and 45.6 inches long (allowing for clearance). The first row of holes would be 3 inches ($0.5k$) from the side of the frame while the second row would be 6 inches ($1k$) from the other side of the frame leaving a distance of 9 inches ($1.5k$) between rows. The first hole in the first row would be at the left end of the frame in the form of a notch, the second would be 10.4 inches ($1.732k$) from the end and the third, fourth, fifth, and sixth would be 20.8, 31.2, 41.6, and 45.4 inches, respectively, from the left end (the interval between the fifth and sixth holes being shortened to keep the sixth hole within the frame). The first hole in the second row would be $0.866k$ or 5.2 inches from the left end, with holes 15.6, 26, 36.4, and 45.4 inches, respectively, from the left end of the frame. A peg would be placed near the right-hand corner of the frame and another opposite it and sunk flush with the edge of the frame so that the one fits into the hole left by the other when the frame is moved.

In applying the nematocide the frame would be laid down with the side adjacent to the first row of holes against the end of the bed and with the left end of the

frame against the left side of the bed. After injecting both rows of holes the frame would be lifted, the holes in the soil covered, and the process repeated 25 times. This would leave 13.5 inches not covered at the far end of the bed. Turning the frame around and placing it against the right side of the bed and injecting the holes would complete the operation. There would have been 27 frames of 11 holes each or 297 injections. At 2 cc per injection, this would amount to 594 cc. For beds not over 46.2 inches wide the last hole in the first row (upper right-hand corner hole) may be omitted.

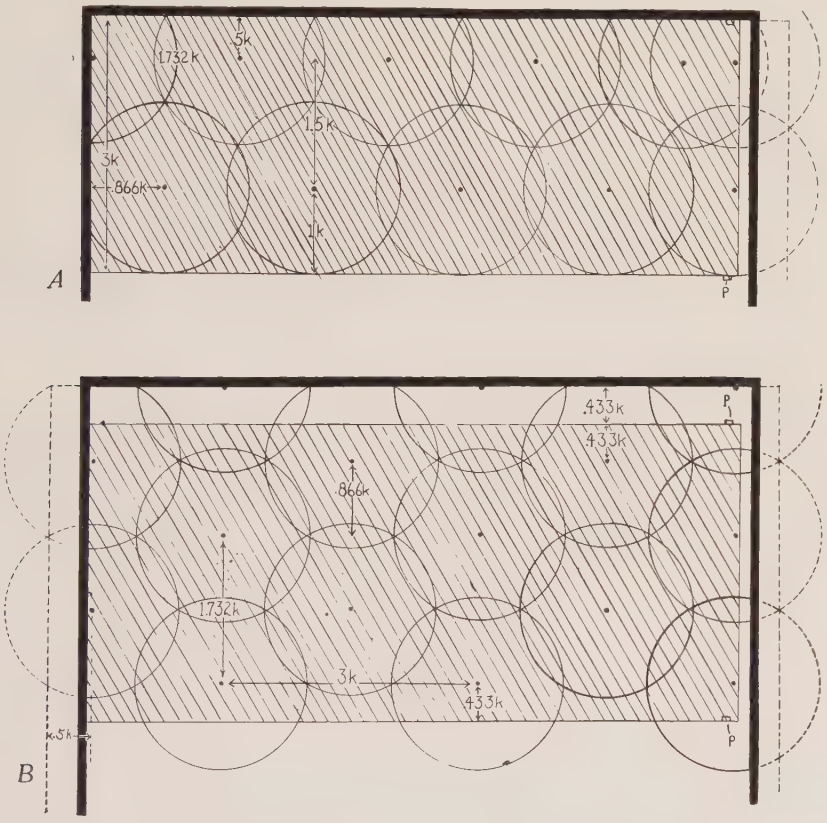

FIG. 1. Transverse frames for the injection of greenhouse beds. A—Frame I. B—Frame II. The sides and end of the bed are indicated by wide black lines, the outline of the frame is shaded. Maximum outline of bed which can be covered by frame is indicated by broken straight lines. Points of injection are indicated by large black dots and the area covered by each injection by a circle around the dot. *p* indicates a peg, *k* stands for killing range.

Frame II. (a) Designed for use in beds 45.6 to 51 inches wide and (b) variants for use in beds 30 to 42 inches wide.—(a) Assuming a bed is 46.5 inches wide by 478.5 inches long, inside measurements, a rectangular frame would be constructed with a width of twice the distance between holes or 20.8 inches (3.464*k*) and a length of 45.6 inches (allowing for clearance) and having 4 longitudinal rows of holes. The first and fourth rows of holes would be 2.6 inches (0.433*k*) from the 2 long sides of the frame while the second and third rows would be 5.2 inches (0.866*k*) from each other and from the adjacent first and fourth rows. The first hole in the

first row would be 0.3 inches ($0.05k$), the second hole 18.3 inches, and the third hole 36.3 inches from the left end of the frame. The holes in the third row would be similarly spaced. In the second and fourth rows the first hole would be 9.3 inches ($1.55k$), the second hole 27.3 inches, and the third hole 45.3 inches from the left end. Pegs would be placed on the right end as described for frame I. In use, this type of frame would be laid down 2.6 inches ($0.433k$) from the end of the bed with the left end of the frame against the left side of the bed. Using a straightedge, a row of 3 injections would be made across the end of the bed, spaced like the holes of the second and fourth rows in the frame. Then the holes of the frame would be

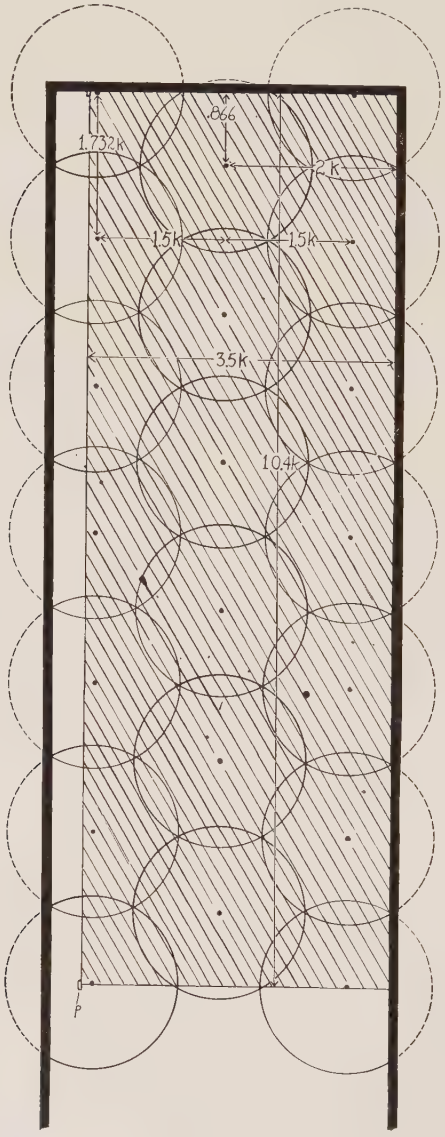

Fig. 2. A longitudinal frame (Frame III) for the injection of greenhouse beds. For explanation see Fig. 1.

injected and the frame lifted and replaced adjacent to the imprint made by its first position. All holes in the frame would again be injected and the process repeated 20 times making a total of 22 frame injections. This would leave 20.9 inches between the last row of injections and the end of the bed. Moving the frame until the first 2 rows of holes overlap the last 2 rows of injections, one would inject the last 2 rows of the frame. Using a straightedge, a row of 3 injections would be made 5.2 inches from the end of the bed, spaced like the first and third rows of holes in the frame and then a marginal row of 3 injections would be made across the end of the bed spaced like the second and fourth rows of holes in the frame. There would have been 22 times 12 plus 6 injections through the frame, plus 6 marginal injections and 3 submarginal injections or a total of 279 injections.

This frame could also be used, without modification, on beds up to 48.3 inches wide (45.3 in. $+ 0.5k$) and by adding a strip 2.7 inches wide to the left end of the frame it could be used on beds up to 51 inches wide. The strip adds $0.5k$ allowing the maximum range for this type of frame.

(b) Reducing the original frame length (45.6 inches) by 6.3 inches at the left end which is placed against the side of the bed, will leave a frame 39.3 inches long capable of covering a bed up to 42 inches wide. A reduction of 9 inches on the left end will leave a frame 36.6 inches long which may be used in beds up to 39.3 inches wide. Further reducing the 39.3 inch frame by 9 inches at the right end would leave a frame 30.3 inches long which would cover beds up to 33 inches wide. An indefinite number of additions and subtractions can be made in order to obtain any specific size of frame desired.

Frame III.—For very narrow beds a slightly different style seems preferable. Again assuming a k value of 6 and with a bed 24 inches wide (22.5 inches inside measurement), a rectangular frame 62.4 inches ($6 \times 1.732k$) long by 21.5 inches wide (slightly over $3.5k$) would be constructed. There would be 3 longitudinal rows of holes, a middle row 12 inches from the right side and 2 side rows each 9 inches ($1.5k$) from the middle row. The first hole in each of the two side rows would be in the form of a notch, subsequent holes being at 10.4 inch ($1.732k$) intervals. The first hole in the middle row would be 5.2 inches ($0.866k$) from the end and the other holes at 10.4 inch intervals. In use this frame would first be placed at the end of the bed as shown in figure 2 and after injection it would be moved until the notches of the first holes of the side rows overlap the points of injection made by the last two holes of these rows. Modifications of this frame will cover beds with inside widths of 18 to 24 inches.

Studies on *Clinostomum*. V. The cyst of the yellow grub of fish (*Clinostomum marginatum*).[1] George W. Hunter, III, Wesleyan University, and H. Clark Dalton, Stanford University.

INTRODUCTION

The cyst walls of larval helminths may be divided roughly into 2 types. The first consists of 2 layers, an inner hyaline sheath, presumably elaborated by the parasite, surrounded by an outer cyst of connective tissue deposited by the host about the inner cyst. The second type consists solely of host connective tissue and is laid down by the host directly about the parasite. The cysts of the yellow grub of fish, *Clinostomum marginatum*, belong to the latter group. Osborn (1911: 356) gives the first description of the structure of this cyst wall. The present paper consists of an elaboration of a preliminary account of a comparative study of *C.*

[1] With the aid of a grant from the Charles Himrod Denison Research Fund in Biology at Wesleyan and a grant-in-aid from Sigma Xi. The assistance of Wanda S. Hunter is gratefully acknowledged.

marginatum cysts reported by Hunter and Dalton (1936) in 4 species of fresh-water fish: the guppy, *Lebistes reticulatus;* the small-mouthed black bass, *Micropterus dolomieu;* the banded sunfish, *Enneacanthus obesus,* and the common sunfish, *Eupomotis gibbosus.*

First evidence of the formation of a cyst in the guppy appears about 3 weeks after the cercaria penetrates. Hunter, Hunter and Kotcher (MS.) note that this host reaction first appears when the connective tissue cells begin to accumulate between the muscle fibers at the ends of the long axis of the parasite. The strands of connective tissue gradually spread around the whole metacercaria, thus developing the cyst wall. Longitudinal sections show that the wall is thickest at the poles and thinnest in the middle of the cyst. About 7 weeks are required after penetration before the cyst is well developed.

It was impossible to study cysts of exactly the same age. However, a comparative study based upon cysts of *approximately* the same age was made. It revealed differences in the amount of connective tissue infiltration that varied with the species of the host. In all cases, however, the cyst walls were composed of fibrous connective tissue cells with their characteristic flattened nuclei. Surrounding this was looser connective tissue which was more noticeable at the ends of the cysts. Melanophores, however, were absent in all cases. In the younger cyst walls studied, fibrocytes, macrophages and lymphocytes were easily distinguished. In the older cyst walls such detailed cellular differentiation had entirely disappeared and usually only a few flattened nuclei were found among the fibers.

THE CYST IN THE GUPPY

The guppy, *L. reticulatus,* was readily infected by *C. marginatum* (Fig. 1). It was not only infected facilely but the metacercariae grew very rapidly. Although the encysted parasites from the guppy were larger than those secured from other hosts, the cyst walls were intermediate in thickness between those of small-mouthed bass and the banded sunfish (Fig. 2, A). The cysts in the guppy appeared

FIG. 1. The guppy, *Lebistes reticulatus,* infected with metacercariae of *Clinostomum marginatum.* About $\times 1\frac{1}{2}$.

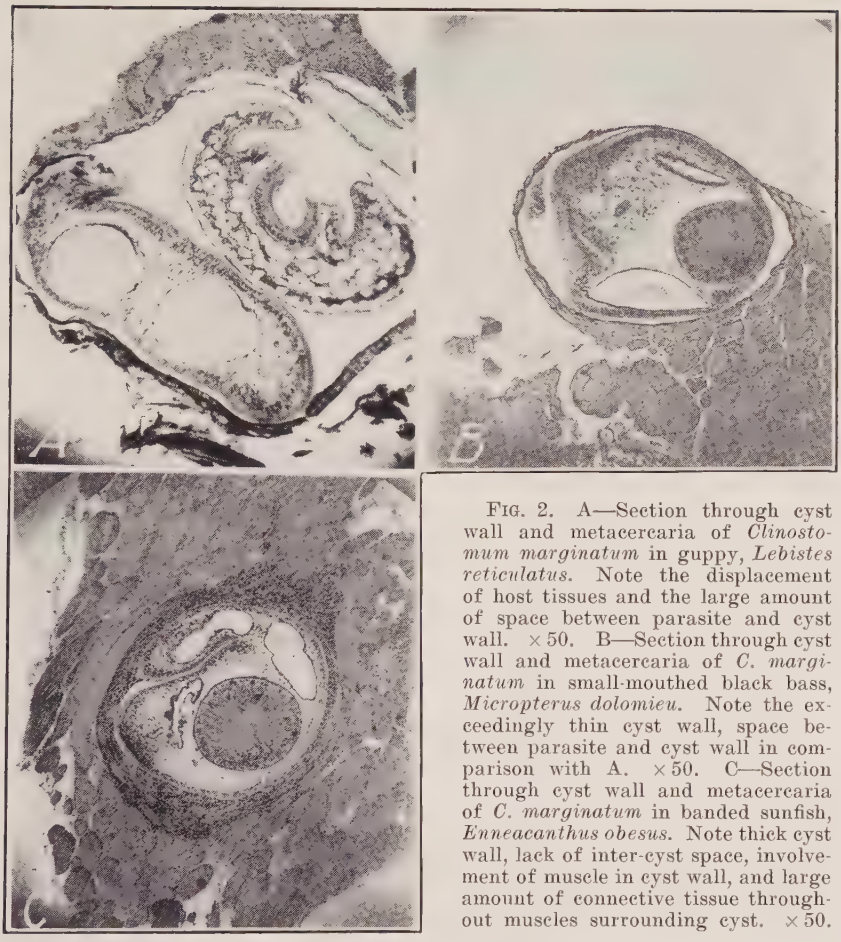

FIG. 2. A—Section through cyst wall and metacercaria of *Clinostomum marginatum* in guppy, *Lebistes reticulatus*. Note the displacement of host tissues and the large amount of space between parasite and cyst wall. ×50. B—Section through cyst wall and metacercaria of *C. marginatum* in small-mouthed black bass, *Micropterus dolomieu*. Note the exceedingly thin cyst wall, space between parasite and cyst wall in comparison with A. ×50. C—Section through cyst wall and metacercaria of *C. marginatum* in banded sunfish, *Enneacanthus obesus*. Note thick cyst wall, lack of inter-cyst space, involvement of muscle in cyst wall, and large amount of connective tissue throughout muscles surrounding cyst. ×50.

to be more independent of the muscle tissue than those studied in the other forms although a few slides clearly showed that the wall may be directly continuous with the perimysium surrounding the muscle bundles. The muscles were often pushed aside and disarranged by the cyst, but the bundles were not separated and surrounded by connective tissue of the cyst as in other species. Displacement of scales was often pronounced in this species. The cyst walls in this host are less tough than those in the bass, being very easily ruptured in fresh material.

THE CYST IN THE BASS

The small-mouthed bass, *M. dolomieu,* is one of the natural hosts for *C. marginatum*. The cyst wall was thinner, although much tougher, in our experimentally infected fish than in the guppy. Also there was much less space between the metacercaria and the cyst wall (Fig. 2, B). In the bass the parasites were more comparable in size to those found in the banded sunfish, being markedly smaller than comparable specimens taken from the guppy.

THE CYST IN THE BANDED SUNFISH

Our experimental infection of the banded sunfish, *E. obesus,* yielded several interesting contrasts. The cyst wall in this form is very thick and dense when

compared with those from the guppy and bass. Muscle bundles were often closely associated with the cyst walls, the connective tissue of the cyst completely cutting them off from their associated muscle bundles and compressing them (Fig. 2, C). Furthermore the muscle bundles immediately surrounding the cyst were thinner and tended to become separated by the infiltrating connective tissue. Thus the amount of connective tissue between the muscle bundles for some distance around the cyst appears greater than was seen in sections of normal tissue. The metacercaria in this host completely fills the cyst even distending the wall in places. One peculiarity was noted in a few cases where melanin appeared to be associated with these cyst walls—a reaction not previously seen for *Clinostomum*. The melanin did not occur, however, in typical melanophores, but rather as granular deposits between cells.

In the experiment only very light infections were obtained, as the banded sunfish proved very resistant to infection. It should also be noted that, to our knowledge, no natural infection of *E. obesus* has ever been reported.

THE CYST IN THE COMMON SUNFISH

The common sunfish, *E. gibbosus*, has often been found infected with *C. marginatum*. The cysts in this form are similar to those in the bass. As in the guppy practically no muscle bundles are incorporated within the cyst walls. These cysts, as in the case of the strigeid black grub, *Uvulifer ambloplitis*, are often found along the septa between the myomeres, suggesting this as the probable route of migration within the host.

Cysts as old as 25 weeks in the banded sunfish, 17 weeks in the bass and 14 weeks in the guppy were similar as far as cellular details of the cysts were concerned. Large nuclei were present and various free cells of the connective tissue could be distinguished. Cysts in all 3 of these species and also in the common sunfish of approximately 57 weeks of age showed decided changes in the cyst wall when compared with the younger cysts. The cellular structure had disappeared, the visible nuclei were much flattened and the walls themselves were very thin in comparison with the younger stages. However, specific host differences centering about the thickness of the cyst wall and involvement of the muscle bundles were still evident.

The older cysts were found to be more easily distorted by fixation. At the ends of the cysts where the heaviest infiltration of connective tissue always occurs, the fibers were sometimes pulled apart. This was noted in *E. gibbosus* especially.

SUMMARY

In conclusion, it may be stated that in the 4 species studied, the type of cyst elaborated by the host is fundamentally the same in all cases of infection with *C. marginatum*. The differences found being in the amount of connective tissue infiltration and in the involvement of the surrounding musculature.

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Preliminary observations of the effect on sheep of pure infestation with the tapeworm, *Moniezia expansa*. D. A. Shorb, U. S. Bureau of Animal Industry.

It is common knowledge that tapeworm infestation of lambs may readily be obtained by permitting them to graze on pastures which have been occupied the year before by tapeworm-infested sheep. However, in acquiring tapeworm infestation the animals usually also become infested with various roundworms and when symptoms of worm infestation appear, it is impossible to determine to what extent they are due to tapeworms.

Until the role of oribatid mites in the life history of *Moniezia* was ascertained by Stunkard (1937) it was impossible to produce experimentally a pure tapeworm infestation in sheep. Stunkard reported that beetle mites, *Galumna* sp., served as intermediate hosts of *Moniezia expansa*, and his reports were confirmed by Stoll (1938) and Krull (1939). These findings have made possible pure infestations under experimental conditions.

In order to determine the effect on sheep of tapeworm infestation uncomplicated by roundworm infestation, cysticercoids recovered from infected beetle mites collected on sheep pastures at the Agricultural Research Center, Beltsville, Maryland, were administered to 4 lambs, each approximately 5 months old; these lambs had not been previously exposed to infestation. The number of experimental animals, the number of cysticercoids administered, and other pertinent data are given in table 1.

Table 1.—*Experimental infestation of lambs with cysticercoids of Moniezia expansa*

Animal designation	Cysticercoids administered			Date eggs first appeared in feces	Date of slaughter of experimental animals	Tapeworms found at necropsy
	Date	Number	Total			
	1938			*1938*	*1938*	*Number*
148	July 20	8	51	Aug. 31	Oct. 25	9
	July 21	[a]				
	July 29	10				
	July 3	15				
	Aug. 6	18				
151	Aug. 12	72	104	Sept. 28	Oct. 25	4
	Aug. 13	32				
145	Aug. 16	160	203	[b]	Oct. 21	0
	Aug. 19	43				
155	Aug. 20	71	110	Sept. 26	Oct. 25	39
	Aug. 22	39				

[a] Fed washings from 6 bucketfuls of grass. The number of cysts present was not known and, therefore, the total number fed to this animal was likewise not known.

[b] No eggs found.

It will be noted from the table that 3 of the 4 experimental animals became infested and that the time intervals between the administration of the cysticercoids and the appearance of tapeworm eggs in the feces were 44, 40, and 37 days, respectively. Lamb 145, which received 203 cysticercoids, apparently did not become infested. The feces of this animal were examined daily from the 35th day after the administration of the cysticercoids until the time of slaughter, but no tapeworm eggs were found at any time, and no tapeworms were present at necropsy.

No definite reason can be assigned at present for the failure of the infestation to become established in this animal.

EFFECT ON HOST

Except for a mild digestive disturbance manifested by the passage of formed but softened feces instead of the usual fecal pellets, the infested animals showed no clinical symptoms of worm infestation. The change in the consistency of the feces occurred 10 days, 12 days, and 1 day before the tapeworm eggs were found. The number of segments passed was not constant and an increase of segments was often accompanied by an increased softness of the feces. The gains in weight of the infested animals was slightly less than those of the uninfested control animals (Fig. 1).

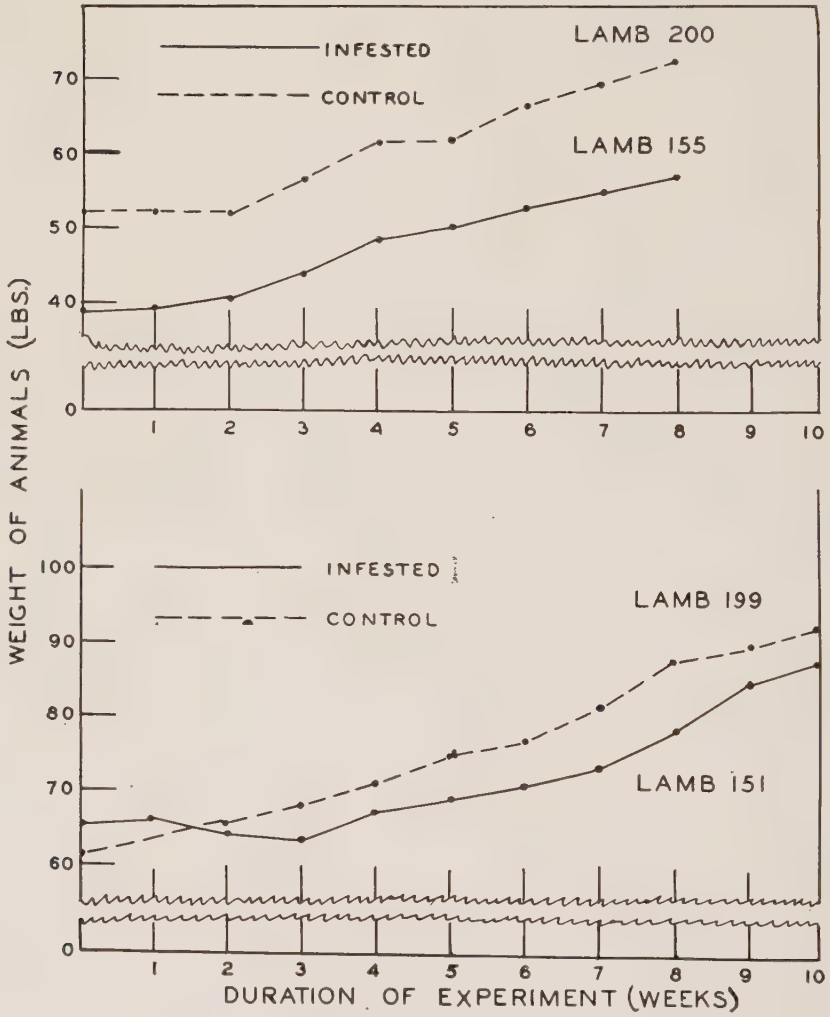

FIG. 1. Weights of infested and control lambs during course of experiments to determine effects of pure infestations of *Moniezia expansa*.

DISCUSSION

Unthriftiness, loss of weight, anemia, and digestive disturbances, manifested by diarrhea, have been attributed to tapeworm infestation. While the animals in the present experiment did not show any of these effects, no definite conclusions on the effect of tapeworm infestation on sheep can be drawn because the number of animals involved was too small. The age of the infested animals probably also affected the results, since under normal conditions of exposure, sheep become infested at much earlier ages than those in the present experiment. Further experiments to determine the effect of tapeworms on sheep are in progress.

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Some new intermediate hosts of the swine stomach worms, *Ascarops strongylina* and *Physocephalus sexalatus*. DALE A. PORTER, U. S. Bureau of Animal Industry.

Alicata (1935, U. S. Dept. Agr. Tech. Bull. 489, pp. 1–96) has listed the following Coleoptera as intermediate hosts of the nematode *Ascarops strongylina*, parasitic in the stomach of swine: *Aphodius rufus, A. castaneus, A. granarius, Gymnopleurus* sp., *Passalus cornutus* and *Scarabaeus* sp. For the closely related swine stomach worm, *Physocephalus sexalatus*, he listed the following beetles which serve as intermediate hosts: *Ataenius cognatus, Canthon laevis, Geotrupes douei, G. stercorarius, G. stercorosus?, Gymnopleurus sturmi, G. sinnatus, Onthophagus bedeli, O. hecate, O. nebulosus, Passalus cornutus, Phanaeus carnifex, P. vindex, Scarabaeus sacer* and *S. variolosus*. To determine the possibility of other species serving as intermediate hosts of these parasites, several collections of dung beetles from hog lots were examined by dissection for the encysted nematode larvae. Duplicate specimens of the beetles were referred for identification to Dr. E. A. Chapin, U. S. National Museum.

A total of 289 dung beetles collected at intervals during 1937 in the vicinity of Moultrie, Georgia, were examined. With the exception of 20 specimens of *Phanaeus vindex*, each of which was infested with larvae of *P. sexalatus*, none of the beetles listed by Alicata were collected by the writer. Encysted third-stage larvae of *Ascarops strongylina* were found in 27 of 145 specimens of *Aphodius lividus* and in 16 of 122 specimens of an unidentified species of *Aphodius*. Third-stage larvae of *P. sexalatus* were found in 4 of 7 specimens of *Copris minutus* and in 4 specimens of *Canthon pilularius*. As far as the writer could determine, the beetles named have not been incriminated before as intermediate hosts of the spirurids in question.

Aphodius lividus and *Aphodius* sp. commonly occurred in large numbers on contaminated pastures and were found to have migrated to manure within 24 hours after it was deposited on a clean pasture. Although not more than 4 larvae of *A. strongylina* were found in the infested beetles, the large number of beetles present at any one time would ensure the presence of large infestations in swine grazing on these pastures. On the other hand, the hosts of *P. sexalatus* were not so commonly found and with the exception of *Phanaeus vindex*, harbored only a few larvae each. All specimens of *P. vindex* examined were found to be infested, having an average of 300 larvae encysted in the body cavity. In an experiment to test the identity of the larvae, a young pig raised free of parasites, was found to

have over 250 specimens of *P. sexalatus* 9 days after being fed 550 encysted larvae, previously recovered from 2 specimens of *P. vindex*. This test would indicate that this beetle is an important intermediate host of this parasite in that only a few such infested beetles would need to be eaten by a pig to produce a sizeable infestation.

North American monogenetic trematodes. IV. The family Polystomatidae (Polystomatoidea). EMMETT W. PRICE, U. S. Bureau of Animal Industry.

This is the fourth installment of a series of papers on the North American monogenetic trematodes, and deals with the family Polystomatidae. This installment is also the beginning of a consideration of the suborder Polyopisthocotylea Odhner. The organization and purpose of this paper are the same as for previous installments (Price, 1937, 1938, 1939).

Suborder POLYOPISTHOCOTYLEA Odhner, 1912

Synonyms.—Polycotyla Blainville, 1828; Octobothrii E. Blanchard, 1847; Eupolycotylea Diesing, 1850; Polycotylea Diesing, 1850.

Diagnosis.—Anterior haptors present, either in form of an oral sucker, or of 2 ventral bothria, or of 2 small suckers opening into oral cavity. Posterior haptor of various shapes, always bearing suckers or clamp-like adhesive organs; hooks present or absent. Eyes rarely present. Genito-intestinal canal always present. Vagina present or absent.

Key to superfamilies of Polyopisthocotylea

Anterior end of body with a pair of small suckers opening into oral cavity. Diclidophoroidea Price

Anterior end of body without suckers opening into oral cavity. Polystomatoidea Price

Superfamily POLYSTOMATOIDEA Price, 1936

Diagnosis.—Anterior haptor in form of an oral sucker, terminal or slightly subterminal. Posterior haptor more or less disc-like, usually with 3 pairs of well developed cup-like suckers (1 pair in *Sphyranura*), with or without appendix-like projection bearing a pair of small suckers and 1 to 3 pairs of hooks; suckers of haptor proper provided with a single hook each, large in Onchocotylidae and small in Polystomatidae. Alimentary tract consisting of short prepharynx, bulbous pharynx, short esophagus, and intestinal branches with or without diverticula or anastomoses. Eyes usually absent. Male and female genital apertures opening to exterior through common opening situated ventrally. Testis single or multiple, postovarial. Vagina double, usually opening laterally. Parasites of amphibians, reptiles, and fishes, rarely in eyes of mammals.

Type family.—Polystomatidae Gamble, 1896.

Key to families of Polystomatoidea

Haptor with appendix-like prolongation ..... Onchocotylidae Stiles and Hassall
Haptor without appendix-like prolongation ..... Polystomatidae Gamble

Family POLYSTOMATIDAE Gamble, 1896

Synonyms.—Polystomidae Carus, 1863; Sphyranuridae Poche, 1926; Dicotylidae Monticelli, 1903.

Diagnosis.—Anterior haptor in form of a more or less well developed oral sucker; posterior haptor disc-like (bilobed in *Sphyranura*), bearing 1 to 3 pairs of

cup-like suckers, with or without large hooks, and with 16 larval hooklets. Intestine consisting of 2 branches, sometimes united posteriorly, with or without diverticula. Eyes usually absent in adults. Common genital aperture ventral, median; cirrus usually with coronet of hooks. Testis single or multiple. Ovary small, dextral or sinistral, pretesticular. Vaginae present or absent. Parasitic in mouth, pharynx, esophagus and urinary bladder of reptiles and amphibians and, rarely, in eyes of aquatic mammals.

Type genus.—*Polystoma* Zeder, 1800.

Key to subfamilies of Polystomatidae

Haptor with 6 suckers ..... Polystomatinae Gamble
Haptor with 2 suckers ..... Sphyranurinae, n. n.

Subfamily Polystomatinae Gamble, 1896

Synonym.—Polystominae Pratt, 1900.

Diagnosis.—Haptor with 6 cup-like suckers. Eyes present or absent. One, two, or many testes. Vaginae, when present, with ventro-lateral openings (vaginae absent in *Oculotrema*).

Type genus.—*Polystoma* Zeder, 1800.

Key to the genera of Polystomatinae

1. Vaginae absent ..... *Oculotrema* Stunkard
   Vaginae present 2
2. Uterus postovarial 3
   Uterus preovarial 4
3. Two testes, lateral ..... *Diplorchis* Ozaki
   Numerous testes or, rarely, one testis, median ..... *Parapolystoma* Ozaki
4. Uterus relatively long, containing many eggs; numerous testes. *Polystoma* Zeder
   Uterus short, usually containing one egg at a time; single testis 5
5. Haptor without large hooks ..... *Neopolystoma*, n. g.
   Haptor with 1 or 2 pairs of large hooks 6
6. One pair of large haptoral hooks ..... *Polystomoidella*, n. g.
   Two pairs of large haptoral hooks ..... *Polystomoides* Ward

Genus *Polystoma* Zeder, 1800

Diagnosis.—Haptor with 1 pair of large hooks. Eyes present or absent. Testes numerous, postovarial, in interintestinal field. Uterus relatively short, preovarial, containing many eggs. Vagina present. Parasitic in urinary bladder of batrachians.

Type species.—*Polystoma integerrimum* (Froelich, 1791) Rudolphi, 1808.

The genus *Polystoma* contains the following species: *Polystoma africanum* Szidat, 1932, from *Bufo regularis* in Africa; *P. gallieni*, n. sp., from *Hyla arborea* var. *meridionalis* and *P. integerrimum* (Froelich, 1791) from *Rana* spp., *Bufo* spp., and *Pelobates cultripes* in Europe; *P. nearcticum* (Paul, 1938), from *Hyla versicolor* and *H. squirella* (new host) in North America; *P. rhacophori* Yamaguti, 1936, from *Rhacophorus schlegeli* var. *arborea* in Japan; and *P. ozakii*, n. sp., from *Rana temporaria ornativentris* in Japan.

Polystoma gallieni, n. sp., is proposed for *Polystoma* sp., which was described by Gallien (1938) from the urinary bladder of *Hyla arborea* var. *meridionalis*. It resembles *P. africanum* Szidat (1932) in lacking prehaptoral intestinal anastomoses, but differs in having more robust hooks and in the absence of extensive invasion of the haptor by the posterior, united, diverticulate portion of the intestine.

Polystoma ozakii, n. sp., is proposed for the form reported as *P. integerrimum* from *Rana temporaria ornativentris* by Ozaki (1935). This species is regarded as distinct from the European *P. integerrimum* on the basis of the intestinal tract. In *P. integerrimum* the intestinal tract shows, apparently consistently, 3 prehaptoral anastomoses (Zeller, 1872; Gallien, 1935), while in *P. ozakii* the intestinal tract shows extensive reticulate anastomosing. This reticulate anastomosing is apparently present in the species reported by Park (1938) as *P. integerrimum* from *R. temporaria* in Korea. If the form reported by Park is identical with *P. ozakii*, as appears to be the case, there are also specific differences in hook structure between *P. ozakii* and the European *P. integerrimum*.

Polystoma nearcticum (Paul, 1938)

Synonym.—Polystoma integerrimum nearcticum Paul, 1935.

This form was named by Paul in 1935 but not described until 1938. The description as given by Paul (1938) is adequate and a redescription is unnecessary in this paper. An examination of the description, as well as of cotype specimens kindly loaned by the collector, has convinced the writer that the differences between this form and the European *P. integerrimum* are more than adequate to justify regarding it as a distinct species instead of a subspecies of *P. integerrimum*. These differences are as follows: The intestine of *P. nearcticum* is more nearly like that of *P. ozakii* than of *P. integerrimum*, being reticulate instead of showing 3 prehaptoral anastomoses; the large haptoral hooks (Fig. 1, A) are less robust than those of *P. integerrimum;* eyespots are absent in the adult form of *P. nearcticum*, and the intestinal tract of the branchial form of *P. nearcticum* shows 4 prehaptoral anastomoses whereas in this form of *P. integerrimum* the intestine is sacculate.

The first record of a species of *Polystoma* from a North American host is that of Riley (1927), and was based on material collected from the urinary bladder of *Hyla versicolor* in Minnesota by Mr. Allen McIntosh. A part of this material has been examined by the present writer through the courtesy of the collector. The specimens were all immature and no specific details could be made out; however, they clearly belong to the genus *Polystoma* and are probably immature specimens of *P. nearcticum*. Recently a single specimen of *P. nearcticum* has been placed at the writer's disposal by Dr. B. B. Brandt; it was collected in North Carolina from the urinary bladder of *Hyla squirella*.

Genus *Parapolystoma* Ozaki, 1935

Diagnosis.—Haptor with or without large hooks. Eyes (?). With single testis or numerous testes. Uterus long, largely postovarial, containing numerous eggs. Vaginae present. Parasitic in urinary bladder of batrachians.

Type species.—Parapolystoma bulliense (Johnston, 1912) Ozaki, 1935.

Two species belong to this genus: *Parapolystoma alluaudi* (de Beauchamp, 1913), from an African batrachian, and *P. bulliense* (Johnston, 1912), from *Hyla lesueurii* and *H. phyllochroa* in Australia.

Genus *Diplorchis* Ozaki, 1931

Diagnosis.—Haptor with 1 pair of large hooks. Eyes present in adults. Two testes, preequatorial, with zones coinciding and fields separate. Uterus long, extending to posterior end of body proper. Parasitic in urinary bladder of batrachians.

Type species.—Diplorchis ranae Ozaki, 1931.

The type and only species was described by Ozaki (1931, 1935) from specimens obtained from the urinary bladder of *Rana rugosa* in Japan.

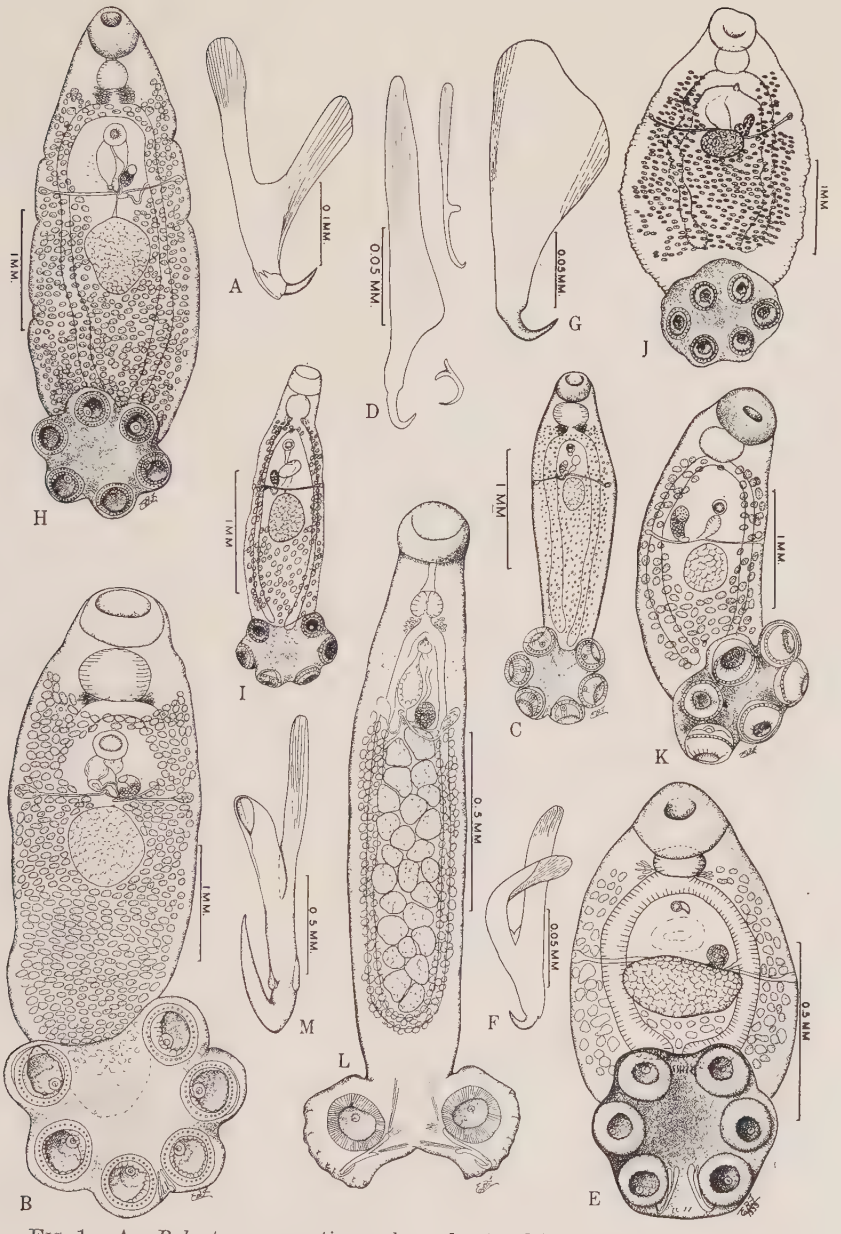

FIG. 1. A—*Polystoma nearcticum*; large haptoral hook. B–D—*Polystomoides coronatum*; B, complete worm, fully mature (*P. albicollis* MacCallum); C, mature worm, not having reached maximum size (*P. digitatum* MacCallum); D, haptoral hooks. E & F—*Polystomoidella oblongum*; E, complete worm (type specimen of *P. hassalli*); F, large haptoral hook. G—*Polystomoidella whartoni*, n. sp.; large haptoral hook. H & I—*Neopolystoma orbiculare*; H, fully mature worm; I, mature worm, not having reached maximum size. J—*Neopolystoma rugosa*; complete worm. K—*Neopolystoma chelodinae*; complete worm. L & M—*Sphyranura osleri*; L, complete specimen; M, large haptoral hook.

Genus *Polystomoides* Ward, 1917

Diagnosis.—Haptor with 2 pairs of large hooks, outer pair larger than inner. Eyes absent in adults. One testis. Uterus short, usually containing one egg at a time. Vitellaria extending into posterior part of body. Vaginae present. Parasitic in mouth, esophagus and nasal passages, usually, and in urinary bladder, rarely, of turtles.

Type species.—*Polystomoides coronatum* (Leidy, 1888) Ozaki, 1935.

The genus *Polystomoides* as here restricted contains 9 species as follows: *Polystomoides coronatum* (Leidy, 1888), from mouth and nostrils of *Amyda ferox, A. spinifera, Pseudemys elegans, Ps. scripta, Chelydra serpentina,* and *Malacoclemmys lesueurii* in the United States; *P. japonicum* Ozaki, 1935, from mouth and esophagus of *Clemmys japonica* in Japan; *P. kachugae* (Stewart, 1914), from urinary bladder of *Kachuga lineata* in India; *P. megaovum* Ozaki, 1936, from urinary bladder of *Geoemyda spengleri* in Japan; *P. microrchis* Fukui and Ogata, 1936, from mouth of *Ocadia sinensis* in Formosa; *P. multifalx* (Stunkard, 1924), from mouth and esophagus of *Pseudemys floridana* and *Ps. hieroglyphica* in the United States; *P. ocadiae* Fukui and Ogata, 1936, from urinary bladder of *Ocadia sinensis* in Formosa; *P. ocellatum* (Rudolphi, 1819), from throat and nasal cavity of *Emys europaea* and *Halichelys atra* in Europe; and *P. oris* Paul, 1938, from oral cavity of *Chrysemys picta* in the United States.

Polystomoides coronatum (Leidy, 1888) Ozaki, 1935
Fig. 1, B–D

Synonyms.—*Polystoma coronatum* Leidy, 1888; *P.* (*Polystomoides*) *coronatum* (Leidy, 1888) Ward, 1917; *P. opacum* Stunkard, 1916; *P.* (*Polystomoides*) *opacum* (Stunkard, 1916) Ward, 1918; *P. megacotyle* Stunkard, 1916; *P.* (*Polystomoides*) *megacotyle* (Stunkard, 1916) Ward, 1918; *P. microcotyle* Stunkard, 1916; *P.* (*Polystomoides*) *microcotyle* (Stunkard, 1916) Ward, 1918; *P. albicollis* MacCallum, 1919; *P. digitatum* MacCallum, 1919.

Description.—Body elongate oval, 3 to 6.4 mm long by 765 μ to 1.6 mm wide at level of vaginal apertures. Oral sucker 133 to 306 μ long by 323 to 765 μ wide. Haptor more or less cordate (sometimes digitate), 970 μ to 1.8 mm wide, bearing 3 pairs of cup-like suckers each 340 to 510 μ in diameter, and armed with 2 pairs of large hooks between posterior pair of suckers, and with 16 larval hooklets distributed as follows: 6 between anterior pair of suckers, 4 between posterior pair of suckers and 1 in each sucker. Outer pair of large hooks 95 to 197 μ long, inner pair 45 to 95 μ long, and larval hooklets 20 to 25 μ long. Pharynx subglobular, 274 to 460 μ long by 304 to 595 μ wide; esophagus very short; intestinal ceca extending to near posterior end of body proper. Genital aperture median, immediately posterior to intestinal bifurcation; cirrus about 133 to 220 μ wide; genital coronet consisting of 14 to 40 hooks, blades 20 to 26 μ long. Testis circular or bluntly oval, 285 to 680 μ long by 190 to 525 μ wide, median, preequatorial. Ovary comma-shaped, 133 to 435 μ long by 64 to 114 μ wide, pretesticular, to right or left of median line. Vitellaria extending from level of base of pharynx to posterior end of body proper, follicles forming band across body at intestinal bifurcation and completely filling posttesticular portion of body. Vaginal apertures ventral, near margins of body, slightly posterior to level of distal pole of ovary. Genitointestinal canal opening into intestine on ovarial side. Eggs oval, 228 to 250 μ long.

Hosts.—*Amyda ferox* (Schneider), *A. spinifera* (LeSueur), *Pseudemys elegans* (Wied), *P. scripta* (Schoepff), *Chelydra serpentina* (Linnaeus), *Malacoclemmys lesueurii*, "spotted turtle" and "terrapin."

Location.—Mouth and nostrils, and (?) urinary bladder.

Distribution.—United States (New York, Massachusetts, North Carolina and Texas) and (?) Canada.

Specimens.—U.S.N.M. Coll. Nos. 46 (cotypes), 30865, 35300, 35569, 35570, 35571, 35572, 35573, 35574, and 35575; Coll. Stunkard; Coll. Harwood.

Since the original description of *Polystoma coronatum* by Leidy (1888), a number of North American polystomes having 2 pairs of large hooks between the posterior pair of haptoral suckers have been described, namely *Polystoma opacum, P. megacotyle* and *P. microcotyle* by Stunkard (1916); *P. albicollis* and *P. digitatum* by MacCallum (1919); *P. multifalx* by Stunkard (1924); *P. stunkardi* by Harwood (1931); and *Polystomoides oris* by Paul (1938). These species were based, for the most part, on the number of hooks of the genital coronet. A comparison of specimens and descriptions of these species indicates that this character is extremely variable and of questionable value in many of the species, especially in the absence of correlated characters. Of the above mentioned species all but *Polystoma multifalx* (syn. *P. stunkardi*) and *P. oris* are regarded as the same as *P. coronatum* (Leidy). A list of the species which are regarded as *P. coronatum,* together with the host, locality, and number of genital hooks, is given in table 1.

TABLE 1.—*Records of specimens regarded as Polystoma coronatum*

Species	Specimens	Host	Locality	Genital hooks
P. coronatum[a] (cotypes)	2	"Terrapin"	Philadelphia, Pa.	32, 33
P. coronatum[b]	1	*Pseudemys scripta*	North Carolina	33
P. opacum[b] (cotype)	1	*Malacoclemmys lesueurii*	Newton, Tex.	33
P. megacotyle[c]	4	*Pseudemys elegans*	Rosenberg, Tex.	33, 38, 40, 40
P. megacotyle[c]	5	*Pseudemys elegans*	Houston, Tex.	30, 32, 32, 34, 34
P. albicollis[a] (cotypes)	3	*Pseudemys elegans*	New York Aquar.	14, 30, 33
P. digitatum[a] (cotypes)	3	*Amyda ferox*	New York Aquar.	24, 31, 32
P. digitatum[a]	2	*Amyda ferox*	New York Aquar.	37, 38
P. digitatum[a]	8	*Amyda ferox*	New York Aquar.	17, 22, 23, 23, 23, 23, 24, 24
P. sp.[a]	3	*Chelydra serpentina*	Woods Hole, Mass.	16, 16, 16
P. sp.[a]	1	"Spotted turtle"	Woods Hole, Mass.	34
P. sp.[a]	4	*Amyda spinifera*	Woods Hole, Mass.	32, 32, 33, 34

[a] Coll. U. S. National Museum.
[b] Coll. Stunkard.
[c] Coll. Harwood.

Each of the collections represented in this table was made on one date and from the same individual. As will be noted, the number of genital hooks varies from 14 to 40, and since no consistent correlated characters have been found they are considered to represent a single species.

Polystomoides multifalx (Stunkard, 1924) Ozaki, 1935

Synonyms.—*Polystoma multifalx* Stunkard, 1924; *P. stunkardi* Harwood, 1931.

This species has been adequately described by Stunkard (1924) and a redescription is unnecessary. Specimens of *P. stunkardi* Harwood from *Pseudemys hieroglyphica* from Oklahoma have been compared with a series of about 25 specimens collected by Mr. A. McIntosh from *Pseudemys floridana* (type host of *P. multifalx*) from southern Florida, and no characters were found which would warrant regarding *P. stunkardi* as different from *P. multifalx.* Harwood (1931) regarded *P. stunkardi* as a distinct species since the number of genital hooks were

fewer in number than in *P. multifalx*. In the series of specimens referred to, the number of genital hooks varied from 82 to 130; this number includes the extremes given for *P. multifalx* (100 to 124) and *P. stunkardi* (92 to 109).

Polystomoides oris Paul, 1938

This species was described by Paul (1938) from specimens collected from the mouth of *Chrysemys picta*. The description is quite complete and nothing of an essential nature can be added. In most respects *P. oris* resembles *P. coronatum* (Leidy); it differs, however, in having diverticulate intestinal ceca.

Polystomoidella, new genus

Diagnosis.—Similar to *Polystomoides*, but having only 1 pair of large haptoral hooks. Parasitic in urinary bladder of turtles.

Type species.—*Polystomoidella oblongum* (Wright, 1879), n. comb.

This genus contains only 2 species, *Polystomoidella oblongum* (Wright) and *P. whartoni*, n. sp., both from North American hosts.

Polystomoidella oblongum (Wright, 1879), n. comb.
Fig. 1, E–F

Synonyms.—*Polystoma oblongum* Wright, 1879; *Polystoma* (*Polystomoides*) *oblongum* (Wright, 1879) Ward, 1918; *P. hassalli* Goto, 1899; *P.* (*P.*) *hassalli* (Goto, 1899) Ward, 1918.

Description.—Body more or less oval, 1.3 to 2.3 mm long by 510 to 616 μ wide at level of vaginal apertures. Oral sucker 95 to 190 μ long by 210 to 360 μ wide, opening subterminal. Haptor more or less cordate, 460 to 715 μ wide, bearing the usual 3 pairs of suckers, each measuring 133 to 190 μ in diameter, and with 1 pair of large hooks 121 to 152 μ long, with deeply incised roots, between posterior pair of suckers; 16 larval hooklets present, arranged as in other polystomes. Pharynx oval, 114 to 190 μ long by 95 to 190 μ wide; esophagus very short; intestinal ceca simple, terminating slightly in front of anterior margin of haptor. Genital aperture median, slightly posterior to intestinal bifurcation; genital coronet with 16 hooks, alternating large and small, the longer about 20 μ and the shorter about 15 μ long. Testis about 250 μ long by 340 μ wide. Ovary comma-shaped, about 76 μ wide. Vitellaria consisting of relatively few follicles, extending from level of base of pharynx to level of anterior margin of haptor. Vaginal apertures lateral, equatorial. Genito-intestinal canal somewhat convoluted, opening into intestine near ovary. Egg oval, 235 μ long by 195 μ wide, according to Wright (1879).

Hosts.—*Sternotherus odoratus* (Latreille), *S. carinatus* (Gray), (?) *Chrysemys picta* (Schneider), *Chelydra serpentina* (Linnaeus), and *Kinosternon pennsylvanicum* (= *K. subrubrum subrubrum* (Lacépède)).

Location.—Urinary bladder.

Distribution.—Canada and United States (Maryland, North Carolina, Texas, Iowa, and Virginia).

Specimens.—U.S.N.M. Helm. Coll. Nos. 1619, 19428, 39576, and 39577.

Polystomoidella oblongum was originally described by Wright (1879) from specimens taken from the urinary bladder of *Aromochelys odoratus* (= *Sternotherus odoratus*) in Canada. It was later reported by Stafford (1900) from *Chelydra serpentina* and again by Stafford (1905) from *Chrysemys picta*. The latter record is open to question since the specimens on which the report was based were from the mouth instead of the urinary bladder.

Polystoma hassalli Goto (1899) is regarded as identical with *Polystomoidella oblongum*, since the large haptoral hooks in both forms have incised roots and are identical in all other respects. Cotype specimens of *P. hassalli* from "*Kinosternon pennsylvanicum*" have been available for study, as well as specimens from *K. sub-*

rubrum collected at Dyke, Va., by Dr. E. A. Chapin and others from the same hosts collected at Piscataway, Md., by Mr. A. McIntosh. Specimens of the form reported by Stunkard (1917) as *P. hassalli* from *Aromochelys carinatus* from Newton, Tex., from *A. odoratus* from Raleigh, N. C., and from "*Kinosternon pennsylvanicum*" from Walker, Iowa, probably are also *P. oblongum.*

Polystomoidella whartoni, n. sp.
Fig. 1, G

Synonyms.—*Polystoma* (*Polystomoides*) *hassalli* of Harwood, 1932; *P.* (*P.*) *oblongum* of Caballero, 1938.

Description.—Body up to 2.7 mm long by about 1 mm wide. Oral sucker about 375 μ in diameter. Pharynx about 100 μ wide; digestive tract similar to that of *P. oblongum.* Haptor about 680 μ in diameter, bearing the usual 6 suckers, each about 200 μ in diameter, 1 pair of large hooks and 16 larval hooklets. Large hooks 148 to 185 μ long, roots not bifid; larval hooklets about 20 μ long, distributed as in other polystomes. Egg 290 μ long by 190 μ wide. Other characters as in *P. oblongum.*

Hosts.—*Kinosternon baurii* Garman; *K. steindachneri* Siebenrock; *K. subrubrum subrubrum* (Lacépède); *K. subrubrum hippocrepis* (Gray); and *K. hirtipes.*

Location.—Urinary bladder.

Distribution.—Canada, United States (Florida and Texas), and Mexico.

Specimens.—U.S.N.M. Helm. Coll. Nos. 41152 (type), 41153 (paratypes), 41154, and 41155.

This species is based upon specimens collected in Florida from *Kinosternon baurii, K. steindachneri,* and *K. subrubrum,* by Dr. G. W. Wharton of Duke University. The specimens upon which Harwood's (1932) report of *P.* (*P.*) *hassalli* from Texas was based were also examined and found to be *P. whartoni.* The report of *P. whartoni* from *Kinosternon hirtipes* in Mexico is based on Caballero's (1938) report of *P.* (*P.*) *oblongum* from that country. The figure of the large haptoral hook of Caballero's specimens shows that it could not be *P. oblongum.*

Polystomoidella whartoni differs from *P. oblongum* mainly in the character of the large haptoral hooks, those of *P. whartoni* being larger and having nonbifid roots, while those of *P. oblongum* are somewhat smaller and have deeply bifid roots.

Neopolystoma, n. g.

Diagnosis.—Similar to *Polystomoides* and *Polystomoidella* except for complete absence of large haptoral hooks. Parasitic in urinary bladder, usually, and nostrils, rarely, of turtles.

Type species.—*Neopolystoma orbiculare* (Stunkard, 1916), n. comb.

This genus contains 6 species; all except *N. exhamatum* (Ozaki) (1935), from the urinary bladder of *Clemmys japonica,* are from North American hosts.

Neopolystoma orbiculare (Stunkard, 1916), n. comb.
Fig. 1. H–I

Synonyms.—*Polystoma orbiculare* Stunkard, 1916; *P.* (*Polystomoides*) *orbiculare* (Stunkard, 1916) Ward, 1918; *P. oblongum* Wright, of Leidy, 1888; *P. troosti* MacCallum, 1919; *P. inerme* MacCallum, 1919; *P. elegans* MacCallum, 1919; *P. spinulosum* MacCallum, 1919; *P. aspidonectis* MacCallum, 1919; *P. floridanum* Stunkard, 1924; *Polystomoides orbiculare* (Stunkard, 1916) Ozaki, 1935.

Description.—Body elongate oval, 2.4 to 5.8 mm long by 318 μ to 1.6 mm wide. Oral sucker 170 to 340 μ long by 272 to 588 μ wide, opening subterminal. Haptor

circular, 700 μ to 1.6 mm wide, bearing the usual 6 suckers and 16 larval hooklets; suckers usually equidistant, about 170 to 425 μ in diameter; larval hooklets about 20 μ long, 6 between anterior pair of suckers, 4 between posterior pair of suckers, and 1 in the bottom of each sucker. Pharynx 187 to 300 μ long by 204 to 390 μ wide; esophagus very short; intestinal ceca simple, extending to near posterior end of body proper. Genital aperture median, near intestinal bifurcation. Genital coronet with 16 hooks, blades about 20 μ long; cirrus pouch about 76 to 148 μ in diameter. Testis oval, 425 μ to 1 mm long by 340 to 680 μ wide, equatorial or slightly preequatorial. Ovary more or less comma-shaped, 120 to 375 μ long by 65 to 170 μ wide, to right or left of median line. Vitellaria extending from level of posterior margin of pharynx to posterior end of body proper, follicles forming band across median field at intestinal bifurcation and filling posttesticular area. Vaginal apertures ventro-lateral, at level of posterior pole of ovary. Genito-intestinal canal opening into intestine on ovarial side. Egg oval, about 228 to 272 μ long by 153 to 170 μ wide.

Hosts.—*Pseudemys scripta* (Schoepff), *P. alabamensis* Baur, (new host), *Chrysemys bellii marginata* (Agassiz), *P. troostii* (Holbrook), *P. elegans* (Wied), *Chrysemys picta* (Schneider), *Amyda ferox* (Schneider), *Malaclemys centrata concentrica* (Shaw), and "terrapin."

Location.—Urinary bladder.

Distribution.—United States (North Carolina, Illinois, Iowa, New York, Minnesota, Oklahoma, Florida, and Texas).

Specimens.—U.S.N.M. Helm. Coll. Nos. 3991, 35101, 35298, 35576, 35577, 35578, 35579, 35580, and 41156.

The above description is based upon specimens in the MacCallum collection. Through the kindness of Dr. H. W. Stunkard, cotypes of *Polystoma orbiculare* and *P. floridanum* have also been available for comparison. This species appears to be fairly variable but the variations appear to be those to be expected in specimens showing different degrees of maturity, and in specimens preserved by different individuals and under different conditions. The specimens described by MacCallum as *P. troosti* had been flattened before fixation and showed enormous distortion; the specimens of *P. elegans* had been exposed to similar conditions; and those of *P. aspidonectis* were not fully mature and showed considerable shrinkage. The specimens of *P. spinulosum* and of *P. inerme* were more mature than those mentioned above, but otherwise showed no essential differences. In the case of *P. floridanum* Stunkard, the specimens appear not to have reached full maturity even though one of the individuals contained an egg. Judging from an examination of about 40 specimens from 8 different hosts, it appears that either the worms commence to produce eggs long before attaining maximum size or that the influence of the host has considerable effect on ultimate size.

Neopolystoma rugosa (MacCallum, 1919), n. comb.
Fig. 1, J

Synonym.—*Polystoma rugosa* MacCallum, 1919.

Description.—Body oval, 2.96 to 3.71 mm long by 1.92 to 1.96 mm wide in equatorial region, flattened dorsoventrally, and with transverse rugae in posterior two-thirds of body. Oral sucker about 425 μ long by 510 μ wide, opening subterminal. Haptor more or less circular, 1.26 to 1.37 mm wide, with customary 3 pairs of cup-like suckers, each sucker 306 to 340 μ in diameter. Pharynx more or less globular, 290 to 340 μ long by 340 to 400 μ wide; esophagus very short; intestinal branches without diverticula, terminating near anterior margin of haptor. Genital aperture at intestinal bifurcation; cirrus pouch about 40 μ in diameter; genital coronet with apparently 14 hooks, the blades of these about 9 μ long.

Testis transversely oval, 340 to 680 μ long by 510 to 765 μ wide, median, slightly preequatorial. Ovary comma-shaped, about 340 μ long by 136 μ wide, to left of median line. Vitellaria extending from level of posterior end of pharynx to near anterior margin of haptor, not meeting anteriorly. Vaginal apertures lateral, about 1.1 mm from anterior end of body. Genito-intestinal canal not observed. Egg oval 360 μ long by 150 μ wide.

Host.—*Amyda ferox* (Schneider).

Location.—Nostrils.

Distribution.—United States (New York Aquarium).

Specimens.—U.S.N.M. Helm. Coll. No. 35581 (cotypes).

This species was described by MacCallum (1919) from 4 specimens collected from a southern soft-shelled turtle; the above description is based upon them.

Stunkard (1924) suspected that this species might be identical with *Polystomoides opacum* (Stunkard) (= *P. coronatum* (Leidy)), since they were from the same host and from the same general location in the host, as well as having a number of characters in common. *Neopolystoma rugosa,* however, is more closely related to *N. orbiculare* (Stunkard), *N. chelodinae* (MacCallum), and *N. terrapenis* (Harwood) than to any of the other species of the genus. In all of these species, 14 to 16 genital hooks are present, but these hooks are larger than in *N. rugosa,* and the vitellaria meet in the median line at the base of the pharynx; in *N. rugosa* the vitellaria do not meet in the median line posterior to the pharynx. In view of these differences *N. rugosa* is retained as a valid species.

Neopolystoma chelodinae (MacCallum, 1919), n. comb.
Fig. 1, K

Synonym.—*Polystoma chelodinae* MacCallum, 1919.

Description.—Body elongate oval, 2.9 mm long by 1.1 mm wide at level of vaginal apertures. Oral sucker 265 μ long by 500 μ wide, opening subterminal. Haptor probably circular (somewhat rectangular in original specimen), 935 μ long by 1.23 mm wide, bearing the usual 3 pairs of suckers, each measuring about 340 μ in diameter; no hooks between posterior pair of suckers. Pharynx wider than long, 340 μ by 425 μ, esophagus not observed; intestinal ceca simple, terminating near anterior margin of haptor. Genital aperture median or nearly so, 250 μ posterior to intestinal bifurcation; genital coronet with 14 hooks, the blades 15 μ long; cirrus pouch about 133 μ wide. Testis globular, equatorial. Ovary comma-shaped, 340 μ long by 170 μ wide, to right of median line. Vitellaria extending from level of base of pharynx to posterior end of body proper, meeting anteriorly at intestinal bifurcation. Vaginal apertures lateral, about 1.36 mm from anterior end of body. Genito-intestinal canal not observed. Eggs not present in available specimens.

Host.—*Chelodina longicollis* (Shaw).

Location.—Urinary bladder.

Distribution.—United States (New York Zoological Park).

Specimen.—U.S.N.M. Helm. Coll. No. 36583 (type).

MacCallum described this species from a single specimen found in the urinary bladder of an Australian long-necked turtle at the New York Zoological Park. It is not known how long the turtle had been in the park, and it is possible that the infection was acquired after its arrival there; it is on this assumption that a description of the species is included here.

Neopolystoma chelodinae is very closely related to *N. orbiculare* (Stunkard) and may actually be the same species. The available specimen is not in good condition and some of the differences which this species shows may be due to faulty technique. Its chief differences from *N. orbiculare* seem to lie in the position of

the genital and vaginal apertures and in the relative size of the oral sucker and pharynx. In *N. chelodinae* the genital and vaginal apertures are much farther posterior than in *N. orbiculare*, and the pharynx of the former is much larger in relation to the oral sucker than in the latter. Whether these characters are of specific value cannot be determined with certainty until better material is available; in the meantime the species is retained as valid.

Neopolystoma terrapenis (Harwood, 1932), n. comb.

Synonym.—*Polystoma* (*Polystomoides*) *terrapenis* Harwood, 1932.

This species has been adequately described by Harwood (1932). The principal difference between *N. terrapenis* and *N. orbiculare* (Stunkard) appears to be in the distribution of vitelline follicles; in *N. terrapenis* the vitelline follicles do not invade the posttesticular field as in *N. orbiculare*. In other respects the two species are very similar, and it is possible that in a large series of specimens it will be found that this character is not constant, and it may be necessary eventually to regard the two forms as identical.

Neopolystoma domitilae (Caballero, 1938), n. comb.

Synonym.—*Polystoma* (*Polystomoides*) *domitilae* Caballero, 1938.

This species was recently described by Caballero (1938) from specimens collected from the urinary bladder of *Chrysemys ornata* in Mexico. This species is apparently more closely related to *N. orbiculare* (Stunkard) than to any of the other North American members of the genus. It differs mainly from *N. orbiculare* in having 19 to 20 genital hooks (16 in *N. orbiculare*) and in the more posterior position of the genital aperture.

Genus *Oculotrema* Stunkard, 1924

Diagnosis.—Haptor without large hooks. No genital hooks. Vitellaria not extending into posterior half of body. Vaginae absent. Parasitic in eye of hippopotamus.

Type species.—*Oculotrema hippopotami* Stunkard, 1924.

This genus contains only the type species which was collected from the eye of a hippopotamus, presumably by Looss at Cairo, Egypt, and described by Stunkard (1924).

Subfamily SPHYRANURINAE, new name

Synonym.—Dicotylinae Monticelli, 1903.

Diagnosis.—Haptor with 2 cup-like suckers. Many testes. Vaginae not opening to exterior.

Type genus.—*Sphyranura* Wright, 1879.

The name Dicotylinae Monticelli is not available for a subfamily as it is not based on any existing genus.

Genus *Sphyranura* Wright, 1879

Diagnosis.—Characters of subfamily.

Type species.—*Sphyranura osleri* Wright, 1879.

Three species have been described as belonging to the genus *Sphyranura*, *S. osleri* Wright (1879), *S. oligorchis* Alvey (1933), and *S. polyorchis* Alvey (1936). Abstract descriptions of these species taken from original sources are given below.

Sphyranura osleri Wright, 1879

Description.—Body 2.6 to 4 mm long by 700 μ wide. Haptor 800 μ long by 1.2 mm wide; large haptoral hooks 240 μ long. Testes 12 to 16 in number. Va-

ginae not opening to exterior, functioning as seminal receptacles. Eggs 400 μ long by 200 μ wide.

Host.—*Necturus maculosus* Rafinesque.

Location.—Skin.

Distribution.—Canada.

Sphyranura oligorchis Alvey, 1933

Description.—Body 2.4 to 3.5 mm long by 300 to 400 μ wide. Haptor 350 to 470 μ long by 700 to 820 μ wide; large haptoral hooks about 260 μ long, without spines. Testes 5 to 7 in number. Seminal receptacles vestigeal, nonfunctional. Eggs 280 to 410 μ long.

Host.—*Necturus maculosus* Rafinesque.

Location.—Skin.

Distribution.—United States (Pennsylvania).

Sphyranura polyorchis Alvey, 1936

Description.—Body 2.4 mm long by 770 μ wide. Haptor 360 to 490 μ long by 700 to 920 μ wide; large haptoral hooks without spines, size not given. Testes 20 to 23 in number. Seminal receptacles vestigeal or absent.

Host, location, and *distribution.*—Not given.

The principal differences between *S. polyorchis* and *S. osleri* are the number of testes (20 to 23 in *S. polyorchis* and 12 to 16 in *S. osleri*) and the absence of spines on the large haptoral hooks. As no specimens of *S. polyorchis* are available for study, it is not possible at this time to pass judgment on its validity. However, an examination of a single specimen, a toto mount, labeled "*Sphyranura osleri,* Zoological Laboratory, University of Toronto," made available through the courtesy of Dr. Justus F. Mueller, Syracuse University, indicates that the large hooks of *S. polyorchis* should be reexamined for "spines." In the specimen (Fig. 1, L) just referred to there were 25 testes and the large haptoral hooks (Fig. 1, M) showed on each side of the blade grooved prominences which are apparently what were regarded by Wright (1879) and by Wright and Macallum (1887) as "teeth." In view of the fact that the number of testes in this specimen is essentially the same as for *S. polyorchis* and the hook characters are those of *S. osleri,* it appears that the differences between the two species as given by Alvey (1936) are probably not valid. Should a reexamination of the hooks of *S. polyorchis* reveal lateral prominences, *S. polyorchis* should be submerged as a synonym of *S. osleri.*

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Description of a plagiorchioid trematode, *Leptophyllum tamiamiensis,* n. sp., from a poisonous snake. Allen McIntosh, U. S. Bureau of Animal Industry.

In this paper a second species of the genus *Leptophyllum* Cohn, 1902, is described. The species is based on three lots of material all from the same snake host, the cotton-mouth or water moccasin. Several specimens of this parasite were received for identification recently from Dr. C. R. Schroeder, New York Zoological Park; the specimens were labeled as being from the rectum and cloaca of an adult male *Agkistrodon piscivorus.* On examination these specimens were recognized as being identical with those of an unidentified species that the writer and his students had collected in March, 1930, from several cotton-mouth moccasins, at the University of Miami, Coral Gables, Florida. Several hundred specimens may be present in a single snake. Of several other species of snakes examined none harbored this parasite. It would appear, therefore, that the adult stage of this distome is specific for *Agkistrodon piscivorus.* A third lot of material was found in searching through the collection of the Bureau of Animal Industry for addi-

tional records of this parasite. These specimens (U.S.N.M. No. 14536) had been collected in August, 1907, by Dr. M. C. Hall from a cotton-mouth moccasin that had died in the National Zoological Park.

Leptophyllum tamiamiensis, n. sp.

Description.—Body oval, 1.2 mm long by 600 μ wide; in less extended specimens than that shown in figure 1, the margin of the body posterior to the acetabu-

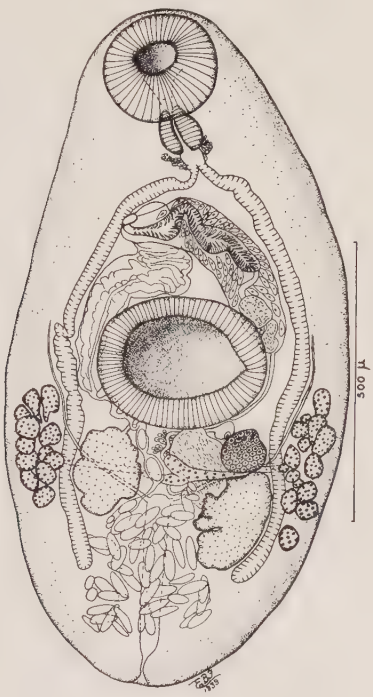

FIG. 1. *Leptophyllum tamiamiensis*, n. sp., ventral aspect.

lum is raised ventrally, giving to the posterior part of the body a spoon-shaped appearance. Cuticula without spines. Oral sucker subterminal, 185 μ by 200 μ; acetabulum slightly postequatorial, 235 μ. by 310 μ. Pharynx 90 μ by 72 μ; esophagus short, 30 μ by 40μ, surrounded by a glandular mass; intestinal crura extending posteriorly beyond vitellaria, ending subterminally. Excretory pore terminal, opening into an elongate bladder which apparently ends near level of ovary. Testes from almost spherical to elongate oval, often with irregular margins, posterior to acetabulum (in type, Fig. 1, abovarial testis 160 μ by 120 μ, adovarial testis 200 μ by 120 μ); testicular fields well separated, with zones partially overlapping. Cirrus sac conspicuously developed, 350 μ by 100 μ, diagonally situated in area between acetabulum and intestinal fork, with base cephalad of ovary near equatorial level of acetabulum; proximal portion of sac enclosing seminal vesicle, distal half containing a very heavily spined cirrus, which in some specimens is protruded. Genital pore about midway between pharynx and acetabulum, lateral to median line. Ovary almost spherical, about 80 μ in diameter, posterior to acetabulum and lateral to median line, anterior to and contiguous with adovarial testis. Seminal receptacle elongate oval, 150 μ to 90 μ, mesal to ovary and posterior to acetabulum; Laurer's canal present; Mehlis' gland and vitelline reservoir medial.

Vitellaria extracecal, in posterior half of body ventral to level of the intestinal ceca, composed of about 12 comparatively large follicles on each side, each follicle about 50 to 70 μ in diameter; anterior limits of vitellaria in zone of posterior rim of acetabulum, posterior limits of vitellaria in zone of testes; vitelline ducts arising from middle of vitellaria, and uniting to form the ventrally placed vitelline reservoir. Uterus composed of few coils, confined, for the most part, between and posterior to the testes, extending posteriorly beyond level of cecal tips. Metraterm unusually large, about the size of the cirrus sac and occupying a similar position on the opposite side of the body; the genital pore is reached by a route dorsal and anterior to distal end of cirrus sac. Eggs elongate oval, 50 to 56 μ by 25 μ, color light brown; few to several hundred present.

Habitat.—Ureters and cloaca of cotton-mouth moccasin, *Agkistrodon piscivorus* (Lacépède).

Type locality.—Tamiami Trail, Everglades, Florida, U. S. A.

Specimens.—U.S.N.M. Helm. Coll. Nos. 44033 (type), 44034 (paratypes), 44035, and 14536.

The genus to which the above species is assigned contains only one other species, the genotype, *Leptophyllum stenocotyle* Cohn, 1902 (Cent. Bakt. [etc.] **32**: 880–882); reported from the rectum of *Herpetodryas fuscus*, a South American snake that had been preserved in alcohol for 10 years before the parasites were removed. The species described here is very similar to the genotype in many respects. However, there appears to be a few points of difference in the writer's material from that described and figured by Cohn to justify the proposing of a second species for the genus *Leptophyllum*. Aside from host difference, Cohn's species has a shorter and more robust cirrus sac, smaller eggs (40 μ by 17 μ), smaller vitelline follicles located dorsal to level of intestinal crura, and with vitelline ducts leading off from the anterior portion of the vitellaria.

Various workers are somewhat in disagreement as to the relationship of *Leptophyllum* to the other genera of the superfamily Plagiorchioidea. Cohn, in proposing the genus, called attention to its nearness to *Enodiotrema* Looss, 1901, and *Styphlodora* Looss, 1899. On account of the heavily armed cirrus (presence of spines not mentioned by Cohn but from his figure they appear to have been present) there appears to be little doubt but that the genus should be assigned to the subfamily Enodiotrematinae Baer, 1924.

Redescription and emendation of the genus *Aproctella* (Filariidae), nematodes from gallinaceous birds. ELOISE B. CRAM, National Institute of Health, U. S. Public Health Service.

Aproctella Cram, 1931, was described in the chapter on internal parasites and parasitic diseases included by H. L. Stoddard in the volume entitled "The Bobwhite Quail—Its Habits, Preservation and Increase," published by Charles Scribner's Sons, New York. The distribution of the volume is limited and as a result it is not available to many parasitologists, especially to those in foreign countries. Moreover, in the original description of the genus, a line was omitted which dealt with the differences in the nature of the cuticle in the dorsal and ventral fields, and in the lateral fields, respectively. Therefore, it is desired to rectify this error, and to make the description of the genus and its type species more readily available.

Aproctella Cram, 1931

Diagnosis (emend.)—Family Filariidae; subfamily Aproctinae. Slender straight worms of approximately the same width throughout whole length; both ends rounded. Head without conspicuous lips or other ornamentation. Mouth

simple, but with papillae. Cuticle of dorsal and ventral fields with coarse longitudinal striations; lateral fields smooth. Anus atrophied or absent. *Male* without caudal alae or caudal papillae; spicules short, subequal, and similar. *Female* with vulva posterior to esophagus, the vagina extending anteriorly from vulva, and the two uteri recurving to run posteriorly. Viviparous; the embryo, unsheathed, forms first as an indefinite globular granular mass, later becoming filiform.

Parasites of abdominal cavity of gallinaceous birds.

Type species.—*Aproctella stoddardi* Cram, 1931.

This genus of nematodes most nearly approximates the genus *Aprocta*, but it differs from the latter in the location of the nematodes in the host; in the fact that the vulva is not in the esophageal region but posterior to it; the vagina instead of immediately running in a posterior direction, is directed anteriorly, the two uteri making the bend and assuming a posteriorly directed course; and in the absence of eggs, unsheathed microfilariae developing in their stead. The division of the cuticle of the body into 4 fields, the dorsal and ventral with coarse longitudinal striations, and the two lateral fields unstriated, is very noticeable in this genus and is not described in *Aprocta*.

Aproctella stoddardi Cram, 1931

Hosts.—Primary: Bobwhite quail (*Colinus virginianus*) as type host; also ruffed grouse (*Bonasa umbellus*); secondary: unknown, probably a biting arthropod.

Location.—Among the viscera of the abdominal cavity.

Description of parasite.—See characters described above in generic diagnosis. *Male* about 6 mm long; esophagus about 320 μ long. Tail short and rounded; spicules similar and subequal, the longer being 75 to 90 μ in length, the shorter 50 to 60 μ in length. Cloacal aperture 63 μ from tail end. *Female* 13 to 15 mm long; esophagus 320 to 335 μ long. Vulva 1.5 mm from head end in a specimen about 13 mm long. No eggs; unsheathed coiled embryos form in uteri. Vagina about 560 μ long, directed anteriorly; the two uteri extend anteriorly almost to the esophagus, then recurve and extend to within 50 μ of posterior end of body. Anus usually not apparent; in a few specimens a rudimentary anus can be seen slightly ventral to the rounded end.

Specimens.—U.S.N.M. Helm. Coll. Nos. 27618 (type) and 28995 (paratype).

Life history.—Unknown.

Distribution.—United States; in bobwhite quail of Southeastern States (Georgia, Florida, South Carolina), specimens submitted by H. L. Stoddard, and in ruffed grouse of New England, specimens submitted by E. E. Tyzzer.

Stoddard found this species much more common in quail from the calcareous coastal "flatwoods" of northern Florida, than in quail from other types of country, a fact which may possibly be linked with the occurrence of the intermediate host in such environment. Of 64 quail examined, 11 per cent were infected; the average number of worms present was approximately 13, the greatest number being 49.

New genera and species of Filarioidea. III. *Sarconema eurycerca* n. gen., n. sp.

EVERETT E. WEHR, U. S. Bureau of Animal Industry.

On May 5, 1924, several specimens of a nematode were collected by E. A. Chapin from the heart muscle of a whistling swan that had died at the National Zoological Park, Washington, D. C. Recently, the writer has received for identification a female specimen of a nematode collected by T. T. Chaddock from the heart muscle of a whistling swan in Wisconsin and many specimens of the same parasite collected by E. R. Quortrop from the whistling swan in Utah. These

nematodes represent a new genus and species for which the name *Sarconema eurycerca* is proposed.

Sarconema, new genus

Diagnosis.—Sexes dimorphic; female considerably larger than male. Body with greatest diameter a short distance back of esophago-intestinal junction. Posterior extremity of both sexes broadly rounded. Cuticle with transverse thickenings. Oral opening circular. Cephalic papillae consisting of 4 pairs of the external circle; internal circle of papillae absent. Esophagus very short, flask-shaped, not divided externally into anterior short, narrow, muscular part and posterior long, broad, glandular part. *Male* with short and approximately equal spicules. Caudal papillae consisting of 4 pairs of preanals, 1 pair of adanals, and a group of 3 marginal papillae. Posterior extremity expanded laterally. *Female* with vulva located a short distance back of head end. Vagina short, stout, and flask-shaped.

Type species.—*Sarconema eurycerca*, new species.

Sarconema eurycerca, new species

Description.—Cuticle with transverse spiral thickenings. Esophagus short, not divided externally into 2 parts, slightly enlarged posteriorly.

Male 11 to 13 mm long and 330 μ wide. Esophagus 321 μ long, slightly constricted in region of nerve ring. Nerve ring 110 μ from anterior end of body. Spicules 140 to 145 μ long, distal end bluntly pointed. Caudal papillae consisting

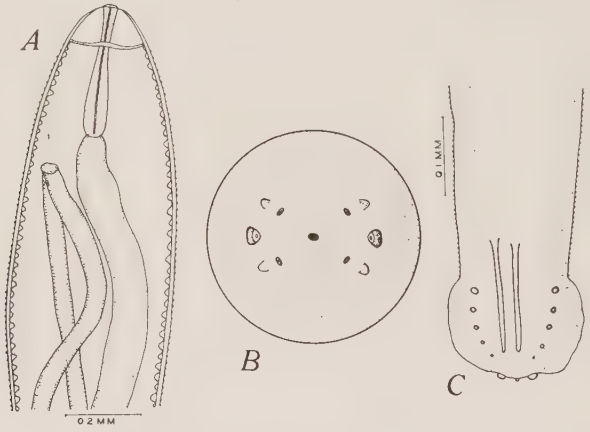

Fig. 1. *Sarconema eurycerca.* A—Cephalic extremity, lateral view. B—Head, *en face* view. C—Posterior extremity of male, ventral view.

of 4 pairs of preanals, 1 pair of adanals, and a group of 3 marginals. Beginning with the very large anterior pair of papillae, each succeeding pair is progressively smaller than the preceding one. Posterior extremity expanded laterally, tip distinctly truncate.

Female 30 to 35 mm long and 750 μ wide at a point a short distance back of vulva. Width of body at level of vulva, 480 μ. Esophagus 375 μ long, flask-shaped. Nerve ring 120 μ and vulva 270 μ from anterior end of body, respectively. Intestine gradually narrowing toward posterior end of body. Vagina flask-shaped, 1.73 mm long, sometimes turning anteriorly a short distance posterior to junction of esophagus and intestine. Uteri parallel, coiling and twisting greatly during their courses through the body. Anus subterminal, 105 μ from tip of posterior extremity. Posterior extremity broadly rounded.

First-stage larva about 260 μ long, with posterior part of body gradually narrowing to a bluntly pointed tip. Cuticle distinctly serrated for the full length of the body.

Host.—Cygnus columbianus.

Location.—Heart muscle.

Distribution.—National Zoological Park, Washington, D. C., Wisconsin and Utah, U. S. A.

Specimens.—U.S.N.M. Helm. Coll. No. 39619 (type) and No. 26143 (paratype).

The genus *Sarconema* must be included in the family Dipetalonematidae Wehr, 1935, and the subfamily Dipetalonematinae Wehr, 1935, because it possesses microfilarioid larvae, the eggs are without chitinous shells, the head of the adult is without circumoral elevation, tridents or pseudonchia, the caudal alae are absent; the body is not swollen at the excretory sinus. This genus resembles *Onchocerca* in having external transverse cuticular thickenings, but differs from the latter in that the body is not swollen at the excretory sinus and the spicules are approximately equal in length.

Helminth parasites of North American semidomesticated and wild ruminants. G. Dikmans, U. S. Bureau of Animal Industry.

There are in the literature a number of scattered records of the occurrence of various kinds of helminth parasites in North American semidomesticated and wild ruminants; there are available also in the U. S. National Museum Helminthological Collection parasites which have not as yet been reported from these hosts. In view of the increased interest manifested in the last few years in the conservation of what is commonly referred to as "wild life," the writer considered that it might be of interest to bring together in one paper the scattered reports and records on the subject mentioned. For convenience the records are presented in tabular form.

From the viewpoint of livestock production it is desirable to have all available information relative to parasites of wild ruminants. As shown in the table, many species are common to wild and domestic ruminants.

Some of the published records are as follows: Hadwen (1916) reported *Fascioloides magna* (*Fasciola magna*) from the black-tailed deer, *Odocoileus columbianus;* he reported also, on the authority of Mr. Kermode, the common liver fluke, *Fasciola hepatica,* from the same species of deer. In 1922, Hadwen reported various cestode and nematode parasites of reindeer, *Rangifer tarandus,* in Alaska, and in the same year he described a new nematode, *Nematodirus tarandi,* from the small intestine of the reindeer. A. E. Cameron (1923, 1924) reported some parasites of the American bison, *Bison bison.* Van Roekel (1929) published on some parasites found in deer in California; the species of deer from which the parasites in question were collected was not given. Fenstermacher and his coworkers (1933, 1934 and 1937), and Wallace (1934) published the results of their examinations for parasites of moose in Northern Minnesota. Swales (1933) published a list of helminth parasites of economically important birds and mammals in Canada and included in it some parasites of wild and semidomesticated ruminants. Later (1934) this author described a new species of nematode from the mountain goat, *Oreamnos americanus;* so far as the writer is aware this is the only helminth parasite recorded from this animal in its natural habitat. Shaw and coworkers (1934) of the Oregon Agricultural Experiment Station recorded a number of parasites from the blacktailed deer, *Odocoileus columbianus.* Roudabush (1936) reported a number of arthropod and helminth parasites found in the American bison, *Bison bison,* in the Wichita National Game Preserve, Cache, Oklahoma. Mills (1936, 1937) mentions some parasites found in elk, *Cervus canadensis,* and in the moun-

TABLE 1.—*Helminth parasites of North American semidomesticated and wild ruminants*[a]

Name of parasite	Hosts												Location of parasite in host
	Alces americana (moose)	*Antilocapra americana* (prong-horn antelope)	*Bison bison* (American bison)	*Cervus canadensis* (elk)	*Odocoileus columbianus* (black-tailed deer)	*Odocoileus hemionus* (mule deer)	*Odocoileus virginianus* (white-tailed deer)	*Oreamnos americanus* (mountain goat)	*Ovibos moschatus* (musk ox)	*Ovis canadensis* (mountain sheep)	*Rangifer arcticus* (caribou)	*Rangifer tarandus* (reindeer)	
Nematodes or round worms													
Gongylonema pulchrum[b]						+	+						Esophagus and rumen
G. verrucosum[b]							+						
Haemonchus contortus[b]			+			+	+						
H. similis							+						
Ostertagia bisonis			+										
O. circumcincta[b]		+		+	+		+		+			+	
O. gruhneri												+	
O. marshalli[b]									+	+		+	
O. mossi							+						Abomasum or fourth stomach
O. occidentalis[b]									+				
O. odocoilei							+						
O. ostertagi[b]			+										
O. trifurcata[b]												+	
Pseudostertagia bullosa		+											
Trichostrongylus axei[b]			+										
Cooperia bisonis			+										
Nematodirella longispiculata	+	+				+						+	
Nematodirus filicollis[b]					+	+							Small intestine
Nematodirus sp.						+	+						
N. tarandi											+	+	

[a] *Bison bison* and *Rangifer tarandus* are regarded as semidomesticated.
[b] Occurs also in domestic ruminants.

TABLE 1 (Continued).—*Helminth parasites of North American semidomesticated and wild ruminants*[a]

Name of parasite	Hosts												Location of parasite in host
	Alces americana (moose)	*Antilocapra americana* (prong-horn antelope)	*Bison bison* (American bison)	*Cervus canadensis* (elk)	*Odocoileus columbianus* (black-tailed deer)	*Odocoileus hemionus* (mule deer)	*Odocoileus virginianus* (white-tailed deer	*Oreamnos americanus* (mountain goat)	*Ovibos moschatus* (musk ox)	*Ovis canadensis* (mountain sheep)	*Rangifer arcticus* (caribou)	*Rangifer tarandus* (reindeer)	
Chabertia ovina[b]					+		+						Large intestine
Eucyathostomum longesubulatum							+						Large intestine
Oesophagostomum radiatum[b]			+										Large intestine
O. venulosum[b]				+	+		+						Large intestine
Skrjabinema oreamni								+					Large intestine
Trichuris sp.						+							Large intestine
Dictyocaulus filaria[b]					+		+						Large intestine
D. viviparus[b]	+		+	+	+	+	+					+	Large intestine
Elaphostrongylus odocoilei					+								Trachea, bronchi and lungs
Pneumostrongylus alpeni							+						Trachea, bronchi and lungs
Protostrongylus coburni							+						Trachea, bronchi and lungs
P. macrotis		+			+	+							Trachea, bronchi and lungs
P. rushi										+			Trachea, bronchi and lungs
P. stilesi										+			Trachea, bronchi and lungs
Elaeophora schneideri						+							Carotid, mesenteric and iliac arteries
Onchocerca cervipedis					+								Subcutaneous tissues of feet

[a] *Bison bison* and *Rangifer tarandus* are regarded as semidomesticated.
[b] Occurs also in domestic ruminants.

TABLE 1 (Concluded).—*Helminth parasites of North American semidomesticated and wild ruminants*[a]

Name of parasite	Hosts												Location of parasite in host
	Alces americana (moose)	*Antilocapra americana* (prong-horn antelope)	*Bison bison* (American bison)	*Cervus canadensis* (elk)	*Odocoileus columbianus* (black-tailed deer)	*Odocoileus hemionus* (mule deer)	*Odocoileus virginianus* (white-tailed deer)	*Oreamnos americanus* (mountain goat)	*Ovibos moschatus* (musk ox)	*Ovis canadensis* (mountain sheep)	*Rangifer arcticus* (caribou)	*Rangifer tarandus* (reindeer)	
Setaria sp.	+		+	+			+				+		Abdominal cavity
Trematodes or flukes													
Paramphistomum cervi[b]	+						+						Rumen or paunch
Cotylophoron cotylophorum[b]							+						Rumen or paunch
Fascioloides magna[b]	+		+	+	+		+						Liver
Cestodes or tapeworms													
Mature													
Moniezia benedeni[b]	+		+							+			Small intestine
Thysanosoma actinioides[b]	+			+	+	+	+						Small intestine
Immature													
Cysticercus lyncis					+	+	+						Heart, lungs
C. krabbei					+							+	Liver, heart
C. tenuicollis[b]	+						+		+	+		+	Liver, mesentery
Echinococcus[b]	+			+	+							+	Liver, lungs

[a] *Bison bison* and *Rangifer tarandus* are regarded as semidomesticated.
[b] Occurs also in domestic ruminants.

tain sheep, *Ovis canadensis,* in the Yellowstone National Park. At various times the writer has published brief notes on the occurrence of helminth parasites in different species of semidomesticated and wild ruminants. A striking feature of the table is the absence of records of the occurrence of the common liver fluke, *Fasciola hepatica,* in North American wild ruminants. The U. S. National Museum Helminthological Collection contains no specimens of this fluke from any of the hosts listed. The only record in the host catalogue maintained in the Zoological Division, of the occurrence of this fluke in a North American wild ruminant is that of Hadwen (1916). An examination of Hadwen's paper shows that the reported occurrence of this fluke in the black-tailed deer, *Odocoileus columbianus,* was on the authority of a Mr. Kermode.

Records of parasites collected from wild and semidomesticated ruminants in the National Zoological Park have been omitted from the table because such animals are not in their natural environment.

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Some helminthic parasites recovered from domesticated animals (excluding equines) in Panama. A. O. Foster, Gorgas Memorial Laboratory, Panama, R. P.

Of the helminths infecting domesticated animals in Panama, only those from equines have been reported with a degree of completeness (Foster, 1936). It has therefore seemed desirable to record the following species which the author has recovered from time to time during recent years from other domesticated animals:

FOWL

*Raillietina echinobothrida, Amoebotaenia sphenoides, *Hymenolepis* sp., **Ascaridia galli, Heterakis gallinae, Subulura brumpti, Cheilospirura hamulosa, Dispharynx spiralis, Strongyloides avium, Capillaria dubia.*

SHEEP

Paramphistomum cervi, Oesophagostomum columbianum, Syngamus sp.

GOAT

Oesophagostomum columbianum, O. venulosum.

CATTLE

Fasciola hepatica, Paramphistomum cervi, Moniezia sp., **Cysticercus bovis, Mecistocirrus digitatus, *Setaria cervi.*

SWINE

**Cysticercus cellulosae, Ascaris lumbricoides suis, *Oesophagostomum dentatum, *Stephanurus dentatus, Necator americanus suillus, *Hyostrongylus rubidus, Metastrongylus salmi, Choerostrongylus pudendotectus, *Ascarops strongylina, *Physocephalus sexalatus, *Macracanthorhynchus hirudinaceous.*

DOG

*Dipylidium caninum, *Toxocara canis, *Ancylostoma caninum, Dirofilaria immitis.*

CAT

Diphyllobothrium mansoni, Dipylidium caninum, Toxocara mystax, Contracaecum sp., *Ancylostoma caninum, Physaloptera praeputialis, P. pacitae* (?).

In the above list, the parasites which are starred (*) were observed by Hall (1928) during his expedition to Panama under the auspices of the Rockefeller Foundation. The others are apparently recorded for the first time from this locality. While the list is by no means complete and is not in any sense the result of an intensive survey, it is probable, nevertheless, that the circumstances under which these parasites were obtained make this a record of the more conspicuous and perhaps more economically important parasites of their respective hosts in this region.

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Helminth parasites collected from deer, *Odocoileus virginianus*, in Florida. A. G. Dinaburg, U. S. Bureau of Animal Industry.

Material from deer killed in Florida in connection with the tick eradication program and sent to the Zoological Division by Dr. J. H. Yoder and Mr. E. C. Bateman of the U. S. Bureau of Animal Industry was examined for helminth parasites. This material consisted of washings from the digestive tract, portions of the rumen fixed in formalin, and of occasional portions of the lungs or liver preserved in borax. The washings from the various portions of the digestive tract were not separated, hence definite locations are not given for the parasites found. Amphistomes were found attached either to the rumen or in the washings, while

liver flukes, *Fascioloides magna,* were found in the liver samples. No parasites were found in the lung samples.

The following tables present the results of the examinations extending over a period from March, 1938, to January, 1939. Since the sample or samples from a given deer sometimes contained more than one kind of parasite, the number of deer infested in each county, as shown in table 2, is greater than the total shown in table 1.

TABLE 1.—*Incidence of helminth parasites in deer from 4 counties in Florida as found by post-mortem examinations*

County	Deer examined	Deer infested	
	Number	Number	Per cent
Orange	175	129	74
Osceolo	53	37	70
Highlands	72	26	36
Glades	8	6	75
Total	308	198	64

TABLE 2.—*Identity of parasites collected and number of deer infested (by counties)*

Kind of parasites	County				
	Orange	Osceolo	High-lands	Glades	Total
Trematoda					
Amphistomes	40	8	14	6	68
Fascioloides magna	22	1	0	0	23
Nematoda					
Ostertagia odocoilei	71	24	9	3	107
Ostertagia sp. (females only)	5	1	4	1	11
Haemonchus contortus	77	32	6	1	116
Haemonchus similis	6	1	1	1	9
Eucyathostomum longesubulatum	6	6	0	0	12
Gongylonema verrucosum	4	0	0	0	4
Capillaria sp. (females only)	3	3	0	0	6
Trichuris sp.	1	0	0	0	1

An examination of the host catalogue in the Zoological Division and of the parasites from the white-tailed deer, *Odocoileus virginianus,* in the U. S. National Museum Helminthological Collection shows that:

Fascioloides magna Bassi, 1875, was determined by Price from a white-tailed deer from South Carolina (U.S.N.M. No. 39504).

Ostertagia odocoilei Dikmans, 1931, was reported by Dikmans (1934, Proc. Helminth. Soc. Wash. **1**: 63) from deer in Pennsylvania, Louisiana, and New York.

Haemonchus contortus (Rudolphi, 1803) Cobb, 1898, has been found by Dikmans in deer from New York (U.S.N.M. No. 33113) and in white-tailed deer from Texas (U.S.N.M. No. 29365).

Haemonchus similis Travassos, 1914, was reported from cattle in Florida, Texas, Louisiana, and Puerto Rico by Dikmans, but has not been previously reported from deer.

Eucyathostomum longesubulatum Molin, 1861, has been reported by Molin (1861, Mem. R. Ist. Veneto Sci. **9**: 427) from the pampas deer (*Blastocerus cam-*

pestris) and the red brocket (*Mazama rufa*) from Brazil, and recently by Cameron (1936, Canad. Jour. Research **14**: 1–5) from the large intestine of the brown wood brocket (*Mazama simplicicornis*) from Trinidad. The present report is the first record of the occurrence of this nematode in *Odocoileus virginianus*. It is also the first record of occurrence of this parasite in the United States.

The domestic cat, a new host for *Thelazia californiensis* Price, 1930 (Nematoda: Thelaziidae). J. R. Douglas, Division of Entomology and Parasitology, University of California, Berkeley.

On February 20, 1939, the author received a collection of nematodes from the eye of a 6-months-old cat from a ranch in the foothills near St. Helena, Napa County, California. This collection consisted of 7 females and 1 male of *Thelazia californiensis* Price (1930, Jour. Parasitol. **17**(2): 112–113). So far as the author has been able to determine this constitutes the first record of this species from the cat. It has been previously reported from the dog many times and once from man, by Kofoid and Williams (1935, Arch. Ophthal. **13**: 176–180). This is, in addition, the first reported natural occurrence of any species of *Thelazia* in the cat, Faust having infected a cat experimentally with *T. callipaeda* Railliet and Henry.

T. californiensis is widely distributed and not uncommon in California. The author has previously reported 43 cases covering a period of 13 years (1938, Jour. Amer. Vet. Med. Assoc. **93**, n. s. **46**(6): 382–384). These cases were distributed over 13 counties, from San Diego County on the Mexican border, to Siskiyou County on the Oregon border.

Buffered solutions in staining helminths. R. Craig and G. M. Spurlock, Division of Entomology and Parasitology, University of California, Berkeley.

The use of buffered solutions in staining tissues has certain very obvious advantages, such as simplification of procedure, saving of labor involved, constant staining of all specimens in a series, improved selectivity, etc. Insofar as the writers are aware, no results have been published on the application of this procedure to helminth tissues.

During the past year the writers have had an opportunity to apply the technique to helminth whole mounts using haematoxylin as the staining agent, and have endeavored to determine the pH range for most selective staining. The solutions were made up in 80 per cent alcohol as recommended by Craig and Wilson (1937, Stain Technol. **12**: 99–109). The following helminths were stained: Cestoda—*Taenia* sp., *Mesocestoides variabilis* Mueller, *Dipylidium caninum* (Linné), *Hymenolepis diminuta* (Rudolphi); Trematoda—*Microcotyle sebastis* Goto (?), *Brachycoelium lynchi* Ingles, *Glypthelmins californiensis* (Cort), *Fasciola hepatica* Linné; Nematoda—*Strongylus vulgaris* (Looss), *S. edentatus* (Looss), *S. equinus* Müller, *Physaloptera* sp., *Aspiculuris tetraptera* (Nitzsch), *Syphacia obvelata* (Rudolphi).

Various pH's were tried with the material noted above. Some of the specimens had been fixed in Bouin's fluid and others in Petrunkevitch's fixative (later formula No. 2; 1933, Science **77**: 117–118) except for *Microcotyle* which had been "fixed" and preserved in 30 per cent alcohol. *Microcotyle* stained hardly at all as would be expected in view of the fixation. The results for the remaining material indicate that the proper pH for Cestoda and Trematoda is in the region of pH 1.2 while for Nematoda it is 1.2 to 1.15. In larger specimens of all 3 groups it is necessary to lower the pH. Since haematoxylin is not too satisfactory a stain for thick whole mounts, experiments are now under way to adapt carmine stains to this technique. This should insure greater transparency of the specimens. In

nematodes, certain structures may be encountered which take stain much more strongly than others, such as the excretory sinus of *Syphacia* or the bursal region of *Physaloptera*. As nematodes have a tendency to collapse or "take air" when transferring from clearing agent to balsam it is usually necessary to puncture them or else transfer them into balsam very gradually.

On thin whole mounts, the differentiation resulting from the use of haematoxylin with this technique is phenomenal, especially if material fixed in Petrunkevitch's fluid is used. It is quite possible to limit staining to nuclei alone so that as in trematodes, all of the nuclei of the acetabulum, etc., may be counted. It is difficult to ruin material since specimens stained at too low a pH can always be restained and likewise specimens overstained can always be destained at the same or a slightly lower pH and $\frac{1}{2}$ the normal concentration of stain. The specimens may be left in stain overnight without staining more deeply, but should be removed sometime during the next day since the quick ripening of haematoxylin by $FeCl_3$ may be carried too far and a brown cytoplasmic stain result.

The writers believe that this technique should be used more widely on helminth whole mounts as well as sections and, from results so far, can sincerely recommend its use.

MINUTES

One hundred ninety-seventh to two hundred fourth meetings

On July 9, 1938, members and other guests assembled at the home of Dr. Paul Bartsch for a delightful supper and informal meeting. Dr. Orsorno, of Colombia, South America, and Dr. J. C. Bequaert, of Harvard University, discussed the South American disease known as "jungle yellow-fever," and related subjects. Dr. G. Steiner described his recent trip to Europe and his visits to various parasitological laboratories on the continent and in England.

The 197th meeting was held October 25, 1938. The following were elected officers: Allen McIntosh, president; E. E. Wehr, vice-president; E. M. Buhrer, corresponding secretary-treasurer; J. T. Lucker, recording secretary. Dr. K. C. Kates and Mr. A. G. Dinaburg were elected to membership and Drs. Carlos de la Torre, I. Perez Vigueras, and A. Moreno were elected corresponding members. Drs. Vigueras and Moreno exhibited a motion picture depicting the life history of *Fasciola hepatica*. Papers were presented by Cort, Otto, Shorb, and Dinaburg.

The 198th meeting was held November 15, 1938. A resolution expressing the loss sustained by the Society in the death of Dr. Maurice C. Hall was read by Dr. Wright, resolution committee chairman, and was adopted. A motion to publish a copy of this resolution in the PROCEEDINGS was unanimously approved. A motion that the date of the 200th meeting be made the occasion of a commemorative dinner was passed. Papers were presented by Steiner, Tyler, Luttermoser, and Rees.

The 199th meeting was held December 20, 1938. Dr. Christie announced that he had been authorized by the Editorial Board to engage a new printer for the PROCEEDINGS. Dr. Cram announced that the Trustees of the Ransom Memorial Fund had decided to aid in defraying the cost of publishing the PROCEEDINGS; $25 from the income of the Fund will be granted annually for this purpose. Dr. Price was nominated to succeed himself as resident vice-president of the Washington Academy of Sciences; he was also elected to the Board of Editors of the PROCEEDINGS, succeeding himself, for a term of 4 years. Papers were presented by the

following visitors: Dr. E. C. O'Roke, Mr. Sterling Brackett, and Dr. L. M. Yutuc. The following members presented papers: Foster, Bartsch, Luttermoser, and Wehr.

A dinner celebrating the 200th meeting was held in the Continental Hotel on January 14, 1939. Dr. J. Fred Denton and Miss Mildred Doss were elected members. The Treasurer reported a balance, on hand January 3, 1939, of $730.80. The program commemorated the founding of the Society and the accomplishments of its pioneer members; Dr. Benj. Schwartz acted as toastmaster. Drs. Cort, Steiner, Stoll, and Bartsch were the other speakers. Registers signed by members and guests were forwarded to Dr. Charles Wardell Stiles and Dr. Albert Hassall.

The 201st meeting was held February 21, 1939. Letters from Dr. Stiles and Dr. C. S. Butler were read. Papers were presented by Otto, Hoffmann, Luttermoser, and Steiner.

The 202nd meeting was held March 21, 1939. Mr. G. M. Spurlock was selected to membership. The Treasurer was authorized to purchase a copy of the "Directory of the Washington Academy of Sciences" for each member of the Society not belonging to the Academy. As a feature of the program, Mr. E. E. McCoy and Mr. H. B. Girth, of the U. S. Bureau of Entomology, described experiments to determine the practicability of utilizing the parasite, *Neoplectana glaseri*, for controlling the Japanese beetle. Dr. Chas. E. Chambliss, president of the Washington Academy of Sciences, addressed the Society, briefly, and two notes were presented by Dr. Wm. Hoffman.

The 203rd meeting was held April 18, 1939. The Corresponding Secretary was authorized to determine the sentiment of members with regard to the advisability of changing the number of meetings per year and the time of meetings. Miss Margart Meyer was elected a member. Papers were presented by Dikmans for J. S. Andrews and by Price, Hoffman, Harwood, and Dikmans.

The 204th meeting was held May 16, 1939. The Corresponding Secretary announced the principal results of a questionnaire sent to members as to their preference regarding the number and time of meetings. Nearly all replies indicated preference for the present schedule, so far as the number of meetings per annum is concerned; a considerable number favored a change in the time of meeting. A motion setting the time of future meetings at 5 P.M. of the third Wednesday of each month (October to May, inclusive) was passed. Papers or demonstrations were presented by Wright, Spindler, Christie, Cram, Dinaburg, Luttermoser, Jacobs, Harwood, McIntosh, and Lucker.

JOHN T. LUCKER,
Recording Secretary.

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